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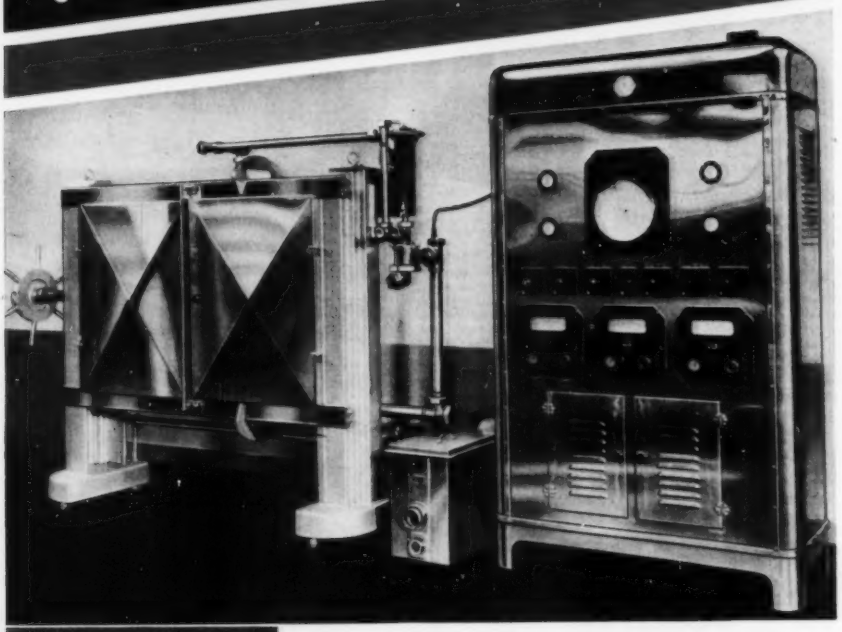

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JOURNAL OF DAIRY SCIENCE

VOLUME XXI DECEMBER, 1938 NUMBER 12

SULFANILAMIDE IN THE TREATMENT OF STREPTOCOCCIC MASTITIS*

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INTRODUCTION

Much interest is being shown at the present time in the use of sulfanilamide in the treatment of streptococcic mastitis of cattle. In the published reports to date, variable results have been obtained by the use of this drug. These facts indicated that further study of this problem should be made.

REVIEW OF LITERATURE

Domagk (4) in 1935 first reported the favorable effect of certain Azo dyes in mice experimentally infected with streptococci. The name "sulfanilamide" was later given to the essential nucleus or effective portion of these dyes by the American Medical Association. Numerous results on the use of sulfanilamide in human medicine have been reported.

Very few controlled experiments with the use of this drug in streptococcic mastitis of cattle have been reported. Allot (1) in November, 1937, reported both clinical and laboratory evidence of improvement in three cows treated with approximately the dose recommended for man, that is, 1 gram for each 20 pounds of body weight. In each instance the case relapsed to approximately its previous condition after treatment was discontinued. Several case reports found in Cattle Breed Journals and Biological Journals have reported favorable results from the use of this drug in doses of 15 to 45 grains (15 grains = 1 gram) 2 to 4 times daily for 3 to 7 days.

Baer and Gunderson (2), reporting on the use of sulfanilamide in the treatment of mastitis, state that streptococci did not appear during treatment but were present after treatment was discontinued on two cows and that streptococci were reduced following treatment on two other cows.

Scholm (8) reported on four cases of streptococcic mastitis treated with sulfanilamide at the California Veterinary Conference, January, 1938.

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* Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper #170.

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These represented slight, moderate, severe and very severe cases of mastitis. The cows averaged approximately 900 pounds each and were first treated with 120 to 150 grains (8 to 10 grams) of sulfanilamide in two equally divided doses daily. The only case that showed any improvement was the one showing slight evidence of mastitis. After a 7-day rest period, the four cases were again treated for 7 days with sulfanilamide in 2 equally divided doses, with the mild case receiving 80 grams daily and the other 3 cases 40 grams daily. The streptococci disappeared from the milk of all quarters in the mild case and from 3 quarters of the moderate case and were reduced in number in the severe and very severe cases. Two weeks after treatment streptococci were again present in all quarters of all the cows treated. No symptoms of intoxication were observed in any of the cows.

Schlotthauer (9) reported on the treatment of one cow for streptococcic mastitis with sulfanilamide at the Intermountain Livestock Sanitary Association meeting in January, 1938. He stated that one 900-pound cow was treated for 3 days with 90 grains (6 grams) daily and showed 1 mg. of the drug in 100 cc. of the milk. She was then treated with 44 grams daily for 4 days. In the neighborhood of 8 hours after the last dose, she showed 5.6 mg. per 100 cc. of the milk. The highest per cent of the drug was found in the normal quarter and much less, or about 1 mg. per 100 cc., in the inflamed quarter. In both treatments there was inhibition of the organisms in the udder but they were not destroyed.

Recently Johnson and Miller (5) have reported on the use of 75 grains (5 grams) of sulfanilamide twice daily for 3 days on 6 cows and ½ ounce (14 grams) twice daily for 8 days on 4 cows afflicted with chronic streptococcic mastitis with no apparent beneficial effects from the treatment. No toxic effects were produced by the treatment.

PURPOSE OF INVESTIGATION

The study at the Idaho station was started in June, 1937, before the above reports were presented, and because of the absence of accurate data then available it seemed advisable to study the following points:

1. To determine how often it would be necessary to administer sulfanilamide to cattle to maintain a reasonably constant level in the blood.
2. To find what dose is necessary to attain a blood concentration in cattle comparable with that obtained in man.
3. To establish the degree of effectiveness of the treatment in streptococcic mastitis, and
4. To determine what unfavorable results, if any, follow its use.

Streptococcic mastitis has been studied in this station herd for the past ten years. During the past fall and winter, a particularly virulent form of the disease has been prevalent. Heifers showing their first clinical symptoms drop in milk production and go off feed. Difficulty is experienced in getting the affected quarters back to normal with past methods of treatment.

DOSE INTERVAL TO MAINTAIN BLOOD LEVEL

In this work, "Stramid," a brand of sulfanilamide marketed by the Alba Pharmeceutical Company, was used. In every instance the material was given by mouth in gelatin capsules. The concentration of unconjugated sulfanilamide in the blood and urine was determined by the method recommended by Marshall, Emerson and Cutting (6). The Marshall, Emerson and Cutting method was adapted to the determination of the concentration of unconjugated sulfanilamide in the milk.

Two cows were used in this phase of the study. A Jersey cow, #107X, was given 5 grams of sulfanilamide for each 100 pounds of body weight. Blood, urine and milk samples were taken at hourly intervals following this dosage and the sulfanilamide concentration determined. Similar data were also taken on a Holstein cow #55X following the last dosage of a 7-day period of treatment. In both instances it was found that sulfanilamide

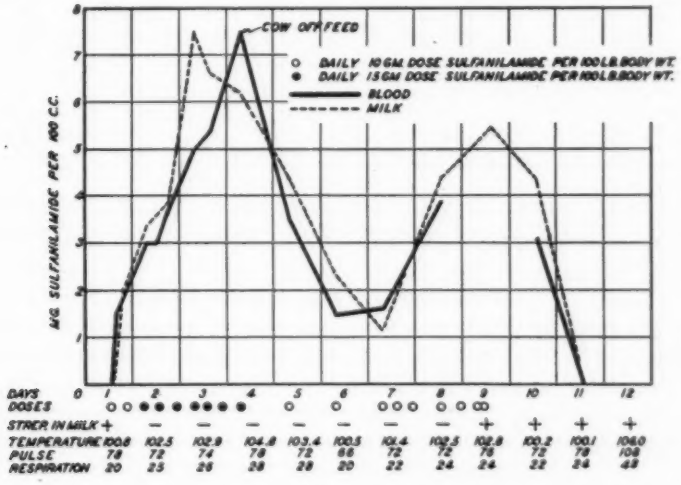

FIGURE 1. Cow #8X. This graph shows the variations in doses given, the fluctuations in the blood and milk levels of sulfanilamide. The presence or absence of hemolytic streptococci in the milk and the effect of the treatment on the temperature, pulse and respiration are also shown. The cow showed symptoms of sulfanilamide poisoning when the blood concentration was between 7 and 8 mg. per 100 cc. This cow died showing cynosis of the voluntary muscles the day following the last shown in the table.

concentration in the blood did not start to drop until about 12 hours after the last dose was administered. Seldom was sulfanilamide detected in the blood or milk 48 hours after the last dosage (See Figures 1 and 2).

The greater persistence of blood level of sulfanilamide in cattle than in man is not surprising, since in ruminants the transfer of material from the rumen is slow, allowing the drug to be presented slowly for absorption

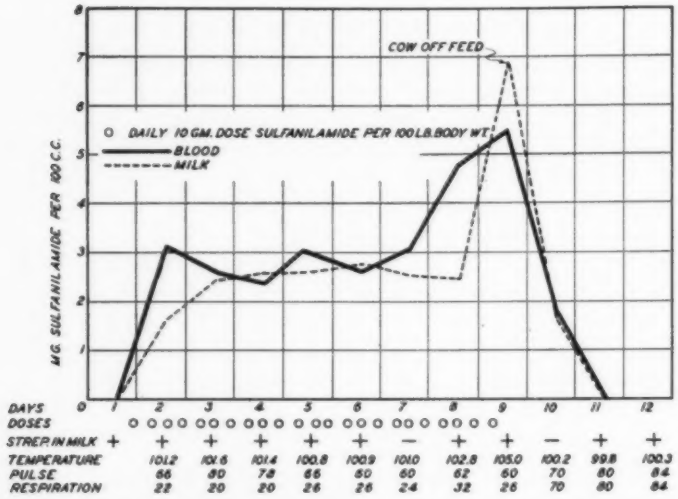
COW OFF FEED
O DAILY 10 GM. DOSE SULFANILAMIDE PER 100 LB. BODY WT.
BLOOD
MILK
MG. SULFANILAMIDE PER 100 C.C.
DAYS 0 1 2 3 4 5 6 7 8 9 10 11 12
DOSES
STREP. IN MILK + + + + + + − + + − + +
TEMPERATURE 101.2 101.6 101.4 100.8 100.9 101.0 102.8 105.0 100.2 99.8 100.3
PULSE 66 60 78 66 60 60 62 60 70 80 84
RESPIRATION 22 20 20 26 26 24 32 26 70 80 84

FIGURE 2. Cow #89X. This shows the general level of sulfanilamide in the blood and milk when a cow receives 10 grams per 100 pounds body weight daily. On the last day of treatment the cow showed symptoms of intoxication. At this time the blood level was about 5.5 mg. and the milk level just under 7 mg. per 100 cc.

to the small intestines. This study would indicate that it was not necessary to repeat the dose of sulfanilamide more often than every 12 hours or twice daily.

BLOOD LEVEL IN RELATION TO DOSE

In the initial trial, where the dosage was at the rate of 5 grams of sulfanilamide to 100 pounds of body weight daily, the blood and milk level remained slightly below 2 mg. per 100 cc. The drug was administered in three or four equally divided doses. This level was far below the 10 to 20 mg. attained in man (6). On subsequent trials, the dose was doubled and trebled that used in man; that is, 10 and 15 grams per 100 pounds body weight. Reference to Figures 1 and 2 show that with these high doses, it was possible to attain blood levels slightly below 8 mg. per 100 cc. Cows regularly go off feed and show other clinical symptoms of sulfanilamide poisoning before the blood level reaches 8 mg. per 100 cc. From this study it would seem impossible to attain blood levels in cattle comparable with that attained in man. This may be due in part to the rapid excretion of this material in the urine or to the possibility of a greater conjugation of sulfanilamide in the blood of cattle.

EFFECTIVENESS OF SULFANILAMIDE IN TREATING STREPTOCOCCIC MASTITIS

Three distinct stages of streptococcic mastitis cases were treated with varying levels of sulfanilamide. Two Jersey cows (#107X and #112X)

had streptococcic infection of about 8 months' duration, one of which showed definite clinical symptoms, were treated with 10 grams per 100 pounds body weight for 7 to 10 days. Streptococci were absent from the milk during most of the period of treatment, but were detected following the termination of treatment. No. 107X had previously been treated with 5 grams per 100 pounds for 9 days without permanently eliminating the streptococci.

Failure to eliminate the causal organisms in these two cases prompted the treatment of two Holstein cows (#89X and #74X). These cows had contracted streptotoccic infection of the udder during the previous 2 months as shown by bacteriological examination of the milk. No clinical evidence of mastitis had developed in either of these cows. They were treated for 8 days with 10 grams per 100 pounds body weight. Streptococci were absent from the milk of #74X during treatment but were present in the milk of #89X. Streptococci were present in the milk of both cows continuously after the termination of treatment. Apparently sulfanilamide even in doses twice that recommended for humans is not effective in eliminating streptococci from the udder of cows affected with non-clinical streptococcic mastitis.

Several cases of acute clinical streptococcic mastitis (#24X, #8X, #85X, #194, #101X, #89X, #128X, #126X, #87X, #103X, #175, and #187) some of which were flare-ups of old chronic cases and others of recent infection were treated with 5 to 10 grams of sulfanilamide per 100 pounds of body weight for periods of 7 to 10 days. Most of these cases had been treated with hot packs, frequent milking, udder ointments, massage, and mild laxatives without eliminating the tendency for the milk to become watery or thick with pus. In some instances the fever, swelling and hardness in the affected quarters were practically eliminated by this standard treatment.

By the end of the sulfanilamide treatment, all cases except one were showing improvement in the consistency of the milk, although many of them were deficient in quantity of milk from the affected quarters.

Treatment with sulfanilamide was successful in restoring normal flow and normal appearance in the milk and the quarter if administered in the early stages when the first symptoms of flaky milk and congestion of the quarter appear. It did not eliminate the streptococci from the udder, nor prevent later acute attacks. This treatment, however, apparently saved many quarters which otherwise would have been lost by reducing the acute infection before the quarter was permanently injured.

Treatment was also effective in restoring normal appearance to the milk of recently infected cows when the condition had developed to the stage where the milk was discolored and watery. However, in these severe cases the affected quarters shrunk to half the normal size and the milk flow was reduced about 50 per cent. One exception to the above (#103X)

showed only partial recovery and relapsed to the previous state after treatment was discontinued.

Use of sulfanilamide resulted in improvement in acute attacks of old chronic cases, but normal milk was not secured in all instances. In one case out of three the milk failed to return to normal appearance.

Five grams of sulfanilamide per 100 pounds of body weight seemed just as effective in producing favorable results as did larger doses.

TOXICITY OF SULFANILAMIDE

Sulfanilamide in doses of 5 grams per 100 pounds body weight, when administered in three equally divided and spaced doses each day over a period up to 12 days, had little or no detrimental effect in 9 of 11 cases treated. This amount did not reduce milk production.

Twice this amount, that is, 10 grams per 100 pounds body weight, after 5 to 7 days' administration, is sufficient to cause distinct toxic effects in the form of general sluggishness, reduction in feed consumption, reduction in milk flow, rough coat and occasionally complete loss of appetite and increased temperature and respiration. (See Figures 1 and 2.)

Higher amounts, that is, 15 grams per 100 pounds body weight daily, for from 1 to 3 days, produce the above-mentioned toxic symptoms accompanied by a precipitate of crystals in the urine. Reduction in milk flow associated with toxic doses was commonly observed. When treatment is discontinued, the milk flow rapidly returns to normal.

One cow (#8X) died the third day following discontinuance of treatment with 10 and 15 grams sulfanilamide per 100 pounds body weight. She became foundered on not more than 10 pounds of grain, developed a diarrhea and died within 36 hours. On post mortem, the liver, spleen and kidneys appeared normal. Definite evidence of acute enteritis was present. All muscular organs were extremely cyanotic, particularly the voluntary muscles which were quite blue on dissection, but soon became normal flesh-colored when exposed to the air. This condition has been found in man associated with sulfanilamide poisoning as recorded by Bigler, Clifton and Werner (3).

One cow (#112X) which was turned out during the last three days of a 10-day period of treatment with 10 grams per 100 pounds body weight, developed an eczema with considerable loose scab production over the head, neck and shoulder and between the limbs and on the udder. The condition seemed to be most severe where there was contact with the stanchion. A similar condition is common in man as reported by Newman and Sharlit (7) when individuals being treated with sulfanilamide are exposed to the direct rays of the sun.

One cow (#126X) died of bloat while on treatment with 5 grams per 100 pounds body weight. She had shown no symptoms of sulfanilamide poison-

ing and did not show any post mortem evidence of poisoning. She is believed to have died of simple bloat.

SUMMARY

1. The blood and milk levels of unconjugated sulfanilamide in cattle were maintained over a period of 12 hours either following an initial dose or after the last dose of a period of treatment. This insures a reasonably constant level of sulfanilamide in the blood of cows that are dosed twice daily at 12-hour intervals.

2. It was possible to attain a level of sulfanilamide in blood and milk slightly under 8 mg. per 100 cc. only when the dose was approximately 10 grams per 100 pounds body weight or twice that recommended for man. Blood levels slightly less than 2 mg. per 100 cc. were attained with a dose comparable with that recommended for man, that is, 5 grams per 100 pounds body weight daily. Both of these levels are below that of 10 mg. per 100 cc. of blood suggested for favorable results in man.

3. Doses of 5, 10, or even 15 gm. per 100 pounds body weight over a period of 3 to 10 days failed to permanently eliminate Beta Hemolytic Streptococci from the udders of cows affected with streptococcic mastitis, regardless of whether the cases were acute or chronic or of short or long duration. Even recently affected non-clinical cases were not freed of the organism.

4. Symptoms of acute streptococcic mastitis such as tenderness, swelling, hardness of the quarter, accompanied by flaky, pussy, or watery milk were relieved in most cases by administering sulfanilamide in doses of 5 to 10 grams per 100 pounds body weight for 7 to 10 days. Five grams per 100 pounds body weight seemed to be as effective as larger doses in relieving clinical symptoms of acute mastitis. The cases treated had failed to respond satisfactorily to the standard treament of applying hot packs, frequent milkings, massages, laxatives and udder ointments.

5. Sulfanilamide poisoning in the form of sluggishness, loss of appetite, reduced milk flow, roughened coat, fever and increased pulse and respiration were produced in 1 to 3 days when the total daily dose was 15 grams per 100 pounds body weight. Five grams per 100 pounds body weight had little or no detrimental effect in 9 of 11 cows treated. The dose should be reduced or eliminated when toxic symptoms appear.

6. One cow died following doses of 10 and 15 grams per 100 pounds body weight with enteritis diarrhea and definite cyanosis of the musculature. Another cow showed extensive eczema when allowed contact with the direct rays of the sun during treatment with 10, and later 5, grams per 100 pounds body weight. There seems to be an individual difference in tolerance of cows to sulfanilamide.

7. Sixteen cows were treated for streptococcic mastitis with sulfanilamide. Eight of nine cases with an initial infection showing acute symptoms gave favorable results with a reduction of inflammation of the udder and restoration of normal-appearing milk. One cow showed symptoms of toxic poisoning and treatment was discontinued. Favorable results were obtained in 6 out of 9 severely affected quarters in 4 old chronic cases. The 3 additional quarters were greatly improved. No improvement was shown in three cases of initial infection where clinical symptoms had not developed.

The bacteriological and chemical phases of this study were conducted with the cooperation of Walter G. Hoge, W. V. Halversen, and V. A. Cherrington of the Department of Bacteriology, University of Idaho. A report covering this phase of the study in more detail will be published as Research Paper No. 169.

Note: Individual case histories of cows treated in this study are available to anyone upon request to the authors.

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AN X-RAY DIFFRACTION ANALYSIS OF CASEIN

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AND

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HISTORICAL INTRODUCTION

X-Ray Studies of Protein Structure

The purpose of x-ray analysis is to determine the arrangement of diffracting units within a crystal unit and to interpret the properties of that crystal in terms of that arrangement. Proteins, in general, are not crystalline in structure, according to Astbury and Lomax (1), and, consequently, typical crystalline effects are not registered on a photographic film when they are subjected to x-ray analysis. Meyer and Mark (2) were the first to apply the principles deduced from the structural arrangement of cellulose to their interpretation of the protein pattern of fibroin. At that time they suggested that fibroin was made up of extended polypeptide chains, built for the most part of alternating glycine and alanine residues. This type of structure has been confirmed for fibered proteins by Astbury (3) who has studied the protein keratin from a number of different sources and under a wide range of conditions. Astbury found that hair keratin existed in two structural forms. The pattern for stretched hair, or beta keratin, could be explained in terms of extended polypeptide chains, whereas the pattern for the unstretched hair or alpha keratin was one of random arrangement. Therefore, this indicated that the chains were in a crumpled state.

Non-fibrous proteins give patterns that cannot be used as successfully for unit cell structure determination as can patterns obtained from fibered proteins. Casein is not a fibered protein, according to Svedberg, Carpenter, and Carpenter (4), but exists as small particles almost spherical in shape. The particle size was detemined by Svedberg (4) by calculation from specific sedimentation velocity data using Stoke's law, under the assumption that the particle was spherical. The value for radius is $(r) = 4.177 \times 10^{-7}$ cm. (41.77 Å). Using the diffusion constant and Einstein's law, particle size was also determined when no assumption was made as to the shape of the particle. The value for r was then found to be $r = 5.994 \times 10^{-7}$ cm. (59.94 Å). The ratio between the two values of rE/rS equals 1.43 and this figure agrees well with the values for hemoglobin, serum albumin, and serum

Received for publication June 3, 1938.

* The data presented in this paper are from a thesis submitted to the Graduate School of the University of Illinois in partial fulfillment of the degree of Doctor of Philosophy, 1937.

globulin that had been determined previously by Svedberg and are known to be non-fibrous.

At times difficulty in interpreting the physical properties of casein and compounds of casein has been encountered. Recently x-ray analysis has been applied to research in the biological fields where valuable contributions have been made by this technique to the knowledge of the structure of certain proteins. In undertaking this study it was considered that x-ray analysis would yield information that could be secured in no other way. If changes in structure of the casein molecule could be correlated with definite treatments, then possibly the properties of this compound could be accounted for by the molecular arrangement.

EXPERIMENTAL PROCEDURE

Methods for Casein Preparation

Pure casein was prepared according to the method of Van Slyke and Baker (5). Although Svedberg, Carpenter, and Carpenter (6) have shown that casein prepared by the Van Slyke and Baker method does not correspond in molecular weight with casein prepared by Hammersten's procedure, nevertheless their results show that casein prepared by either method is a mixture of protein molecules of varying molecular weights. The main constituent of Van Slyke's and Baker's casein, according to Svedberg (6), corresponds to a molecular weight of 100,300, whereas approximately one-third of the casein prepared by Hammersten's method has a molecular weight of 375,000.

Casein was also prepared by hydrochloric acid precipitation from fat-free milk by adjustment of the pH to 4.6. Casein products prepared by rennin coagulation, ultra-filtration, ultra-centrifuging, and electric deposition were also used.

Various addition compounds of casein were prepared from the Van Slyke-Baker casein. The following chemicals were used for their preparation: NaOH, Na_2CO_3, NH_4OH, $Na_2B_4O_7$, and 40 per cent formaldehyde. The procedures followed were those published by Sutermeister (7).

X-Ray Analysis

For x-ray analysis the usual diffraction technique was used. X-rays were generated in a Philips metallix type tube, using a copper target. The majority of exposures were taken when the tube was being operated at 35 KV and 25 Ma. X-rays from an iron target were also tried. The tube was then operated at 18 KV and 15 Ma. The powder method of Hull, Debye, and Scherrer was used with the sample mounted directly in front of the .025 inch lead pin hole. The exposures lasted from 3 to 6 hours. Each pattern was registered on a flat film held in a casette 5 cm. from the sample. Figure 1 illustrates, by a diagrammatic drawing, the laboratory technique that was

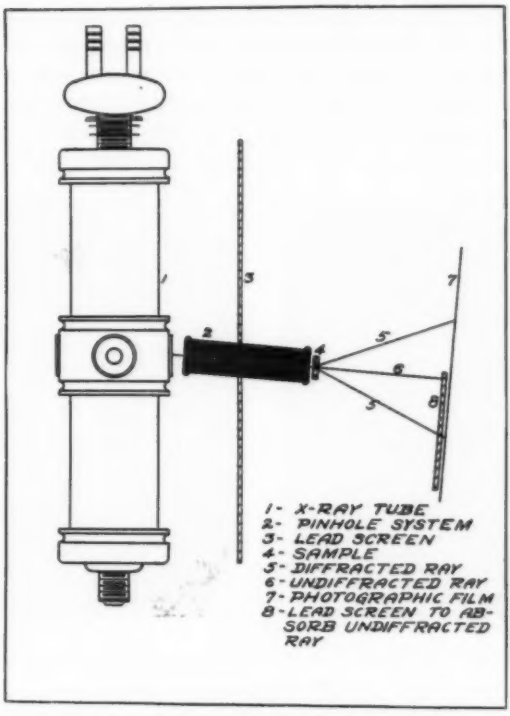

FIG. 1. Diagrammatic sketch of x-ray tube.

used for the x-ray diffraction analysis of the substances studied. The same diffraction pattern was obtained regardless of whether a thin sample (.1 to 1 mm.) of dried casein was used or whether the sample was first ground to a powder with mortar and pestle. When the materials were not dried broad diffuse halos were obtained instead of the characteristic pattern of the dried casein samples.

Interpretations of the powder patterns obtained from the various substances were made by the use of the Bragg equation, $n\lambda = 2d \sin \theta$. The radii of the diffraction rings were determined and then by appropriate calculations and substitution in the above equation the "d" values, or distances between diffraction units or planes, were calculated. The "d" values are specific and characteristic for each substance. This technique, therefore, serves as a ready means for analysis and identification of materials.

EXPERIMENTAL RESULTS AND DISCUSSION

X-Ray Diffraction Analysis of Casein

Pure casein, made by the Van Slyke and Baker (5) method, was the first

to be subjected to x-ray analysis by the pin-hole method. Following this, caseins made by the procedures previously described were analyzed. The diffraction patterns obtained from each of these products had two halos characteristic of complex proteins whose component groups are apparently not arranged in a geometric fashion, but whose arrangements are entirely ones of random. Although Figures 2 and 3 illustrate only the patterns ob-

FIG. 2. Pure casein.

FIG. 3. Casein coagulated by rennin.

tained from pure casein and casein coagulated by rennin, the patterns from the other caseins are almost identical. The calculated spacings for d_1 and d_2 of these caseins are of the same order of magnitude, which is evidence of their similarity. The values are listed in the following table.

TABLE 1

Spacing values for caseins

Spacing	Pure casein Van Slyke and Baker	Crude com. casein ppt. by HCl	Casein coagulated by rennin	Casein from ultra filter	Casein from ultra centrifuge	Casein deposited by elect. current
d_1	9.9 Å	9.8 Å	9.9 Å	9.9 Å	9.84 Å	9.7 Å
d_2	4.66 Å	4.60 Å	4.62 Å	4.60 Å	4.65 Å	4.60 Å

Although the above products were prepared by entirely different procedures the method of preparation had no marked influence on the internal arrangement and molecular structure of the caseins, as determined by x-ray diffraction analysis.

The diffraction pattern obtained from casein (1, 8) is typical of non-fibrous proteins which are without sufficient orientation or number of inter-

ferences to lend themselves readily to accurate analysis, and as a result no satisfactory solution of structure has been developed. The probable explanation of the lack of structural organization in casein and other proteins of a like nature, according to Clark and Schaad (9), is due to the mutual attraction of the large number of polar groups that probably occur in a complex protein structure. The polar groups are apparently distributed at irregular intervals in both side chains and along main chains; hence the structural design is not one of a regular geometric pattern but is one due to the chemical nature of the material.

Emil Fischer was the first to advance the hypothesis that proteins are essentially a synthesis of polypeptide chains, and since the work of Meyer and Mark (2) x-ray diffraction data of protein structure has been interpreted on the assumption that proteins are built up of polypeptide chains which are either in an extended condition, as in stretched hair, or in a crumpled state, as in unstretched hair. X-ray data, as interpreted by Astbury (10), have also shown that side chains extend from the main chain at regular intervals and these serve as definite diffraction units in determining the perpendicular distance, in the same plane, between the polypeptide chains. This spacing is exceedingly varied since it seems to depend to a large extent on the amount of water held by the protein. For the majority of proteins this value has been calculated to be from 9.5 Å to 10.5 Å, although Clark and Schaad (9) have found it to vary from 10.4 Å to 17 Å for certain proteins, depending on the amount of water bound by the protein. On the other hand, it can be seen from Table 1 that this spacing was very uniform in the different casein samples analyzed.

In addition to this 9.8 Å spacing which is always present in diffraction patterns of proteins, another one appears which represents the perpendicular distance or thickness between planes of polypeptide chains. This value is rather uniform for all proteins, being for the majority 4.6 Å. The casein samples showed no exception for this spacing, and all samples were very uniform in this spacing value. Secondary valence forces are assumed to be responsible for the holding together of the polypeptide chains in the protein grid (10, 11, 12).

In the production of the peptide linkage there is a combination of the amino group of one amino acid with the carboxyl group of another, to yield the CONH group. Apparently the alternation of CONH and RCH groups determines the repeating patterns of proteins. The characteristic identity period for silk, hair, and other proteins has been of the magnitude of 3.5 Å (10). A spacing at 3.08 Å has been found to be present in cheese but not in casein.

Although the peptide linkage seems to be the most common and prominent type of binding proteins, Abderhalden's diketopiperazine hypothesis

has been used to explain a few peculiar reactions of proteins to certain treatments and reagents.

The Reaction of Casein to Certain Alkaline Reagents

In order to determine the effect of alkalis on casein, alkaline caseinate compounds were prepared as previously described. Each of these gave the same x-ray diffraction pattern and, upon calculation, the "d" spacings were approximately the same as for uncombined casein. This would indicate, then, that the preparation of sodium caseinate glues and plastics, although bringing about decided changes in physical and chemical properties of casein, does not produce any characteristic or definite change in molecular structure. Apparently only surface reactions are involved. However, when NaOH was added to casein and the sodium caseinate heated to boiling, a marked change in color appeared. This reaction produced a decided change in the diffraction spacings, as shown in the table below. Apparently there was a tendency for shrinkage and a closer packing of the peptide linkages.

TABLE 2

Spacing values for alkaline compounds of casein

Spacing	Casein NaOH	Casein NH_4OH	Casein $Na_2B_4O_7$	Casein Na_2CO_3	Casein NaOH Boiled
d_1	9.78 Å	9.84 Å	9.78 Å	9.80 Å	9.34 Å
d_2	4.61 Å	4.67 Å	4.62 Å	4.60 Å	4.45 Å

X-ray diffraction patterns for two of the above preparations are illustrated by Figures 4 and 5. Each shows characteristic halos of non-fibrous,

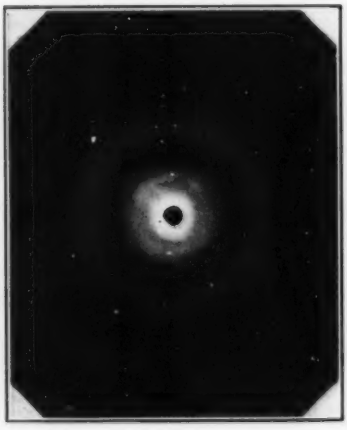

FIG. 4. Sodium caseinate (NaOH + casein).

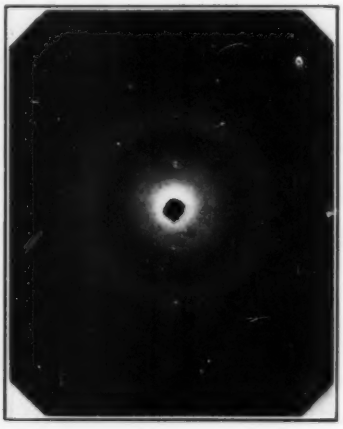

FIG. 5. Boiled sodium caseinate.

non-crystalline proteins. Astbury and co-workers (13) assert that denaturation of proteins brings about a tendency for a fibering of proteins as they are most stable and most insoluble in this condition. According to them, "the denatured state is essentially a fibered one, inasmuch as it always consists of peptide chains often fully extended and aggregated after coagulation, as in fibroin."

Although casein was denatured by boiling in strong alkaline medium, orientation could not be observed. Long threads were produced, dried under tension, and mounted either parallel with, or perpendicular to, the plane of x-rays, but no orientation was noticed. Coagulation of casein by rennin also denatures this protein. However, no fibering or orientation occurs, even though the protein is stretched into long threads.

The reaction of alkalis and acids with casein that brings about a change in the physical properties of casein but not a change in the molecular structure might be explained by assuming that proteins, in general, are amphoteric electrolytes (14). They combine with anions on the acid side of the isoelectric point and with cations on the alkaline side. The isoelectric point of casein is at pH 4.7. In making sodium caseinate the reaction occurs at pH 10, or greater. Under these conditions the reaction would occur at the COOH radical. The sodium caseinate that is formed could then dissociate into a protein anion and a Na^+ cation. On the acid side of the isoelectric point the amino group of the protein molecule behaves like ammonia in its ability to add an acid. The hydrochloride that is formed could then dissociate into a cation and a Cl^- anion. Assuming then that the casein would react as colloidal particles, there are undoubtedly numerous COOH and NH_2 groups that would react in the manner described, producing changes in physical properties but no profound molecular structural rearrangement due to the reaction.

The Reaction of Casein and Formaldehyde

The chemical reaction that occurs between casein and formaldehyde is decidedly interesting and, from an industrial viewpoint, highly valuable, for on this reaction is based the production of goods of the casein plastic industry. When a weak solution of formaldehyde (3–5 per cent) is added to a sheet of casein a hardening or denaturation reaction occurs that is solely characteristic of formaldehyde. At first, like the alkali reactions, this appears to be only a surface reaction, since the hardening occurs very shortly after the casein is in contact with the formalin solution. However, the reaction continues and proceeds at a more or less constant rate, according to Henley (15). This investigator worked with serum proteins and found that the formol reaction was of the second order; that is, for a given reaction to occur, the time required is inversely proportional to the original concentration. Although the reaction of formaldehyde on casein rapidly alters

the physical properties of the casein, no marked internal change in structure occurs. However, at the end of five days in 40 per cent formalin a sample of ultra-filter casein showed a new strong diffraction interference that is characteristic of the reaction of formaldehyde and casein.

It was first considered that the presence of the new diffraction interference would aid in interpreting the casein formaldehyde reaction, but further work showed this new line to be due to polymerized formalin that had crystallized in the casein during the reaction (16).

Although formalin produces decided physical changes in casein, it has not been possible by x-ray diffraction analysis, as yet, to show marked molecular structural changes, occurring as a result of the reaction.

SUMMARY

By x-ray diffraction analysis an attempt has been made to interpret changes that were produced by the action of acids, alkalis and formaldehyde on casein, as well as, attempting to distinguish differences in casein prepared by various methods.

No marked differences were found in the casein samples prepared by various procedures. Only by vigorous boiling in the presence of NaOH were marked changes in structure observed due to chemical treatment.

The two halos produced by casein upon x-ray diffraction analysis were typical of non-fibrous proteins. The halos were at spacings of approximately 4.6 Å and 9.8 Å which correspond respectively to the vertical distance between layers of polypeptide chains and the longitudinal distance of side chains from the main chain. These values agree well with other non-fibrous proteins that have been subjected to x-ray analysis.

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AN X-RAY DIFFRACTION ANALYSIS OF CHEDDAR CHEESE

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INTRODUCTION

The determination of chemical changes occurring in cheddar cheese during ripening has been the subject of research by numerous investigators.

Van Slyke and Hart (1) in 1902 considered the first step in cheese ripening was the peptic digestion of paracasein. They found that as the cheese aged there was an increase in water soluble nitrogenous products.

Later these same investigators (2) studied the individual proteolytic compounds liberated from the aged cheddar cheese. After 214 days at 15.5° C. (60° F.) they found that 15 grams of CO_2 had been liberated, which was .5 per cent of the weight of the fresh cheese. In addition, tyrosine, oxyphenolethylamine, arginine in traces, histidine, lysine, guanidine, and putresine in traces were also identified as hydrolytic products of cheddar cheese ripening.

Kelley (3) showed that the protein of cheddar cheese is hydrolyzed at a fairly uniform rate, and that at 90 days approximately 20 per cent of the protein was water soluble.

Lane and Hammer (4) in studying the rate of protein hydrolysis in cheese made from raw milk, and cheese made from pasteurized milk, concluded that cheese made from raw milk ripened more rapidly as measured by protein hydrolysis.

The purpose of the present investigation was to study the chemical changes occurring in cheddar cheese, both by chemical analysis and by the x-ray diffraction technique.

EXPERIMENTAL PROCEDURE

X-Ray Diffraction Analysis

The procedure followed in securing the x-ray patterns of the experimental samples has been described in the preceding paper.

The cheese samples used for diffraction analysis were prepared by cutting a thin slice of the cheese approximately .5 mm. thick and then drying it at 37° C. for 12 to 24 hours. The samples were then washed with ether

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* The data presented in this paper are from a thesis submitted to the Graduate School of the University of Illinois in partial fulfillment of the degree of Doctor of Philosophy, 1937.

to remove the ether soluble material. When cheese samples were not dried, broader and less sharply defined rings were obtained. When the fat was not removed by ether extraction, lines due to liquid fat were obtained.

The pure amino acids which were used for the standard patterns in identifying the amino acids liberated in the cheese were obtained through the courtesy of Professor W. C. Rose, of the Division of Biochemistry, University of Illinois.

Methods for Chemical Analysis of Cheese

Chemical analyses of the cheeses were made after one day, one week, and at monthly intervals thereafter until the cheese was 180 days old.

The methods used and the procedure followed were much the same as those used by Kelley (3). Hydrogen ion concentration measurements were made, using a L & N Quinhydrone pH Indicator with a saturated Calomel electrode. The technique followed was similar to that which Brown and Price (5) found to be satisfactory.

N-Butyl Alcohol Extraction of Ripened Cheese

A modification of Dakin's (6) method of n-butyl alcohol extraction of hydrolyzed protein was made to secure mono-amino acids from ripened cheese. The cheese samples after being grated to shreds were extracted with petroleum ether until practically fat free. Two hundred to five hundred grams of cheese were extracted four times with one liter of hydrated n-butyl alcohol. The cheese and butyl alcohol were mixed by shaking on a mechanical shaker one to two hours for each extraction. The alcohol extract was filtered and then evaporated under reduced pressure at 30° C. to about one-fourth its volume. By that time the mono-amino acids had crystallized in the dry butyl alcohol so that they could be filtered off, dried, weighed, and analyzed by x-ray diffraction methods.

Manufacturing Procedure for Cheese Used for Chemical and X-ray Analysis

The manufacturing procedure for the two lots of cheddar cheese used in the ripening study was varied in such a way as to produce one lot which would cure slowly and the other lot which would ripen rapidly. This was done by varying the amount and rate of acid development. Lot 1115 was the slow ripening cheese and lot 1120 was the rapid curing cheese. Both lots were made from pasteurized milk of low bacterial count. The manufacturing data for the cheese is listed in Table 1.

During the manufacturing process samples of the cheese were taken for x-ray analysis. Also at definite intervals throughout the ripening period, the cheese was subjected to x-ray examination and chemical analysis in order to correlate the changes in the patterns that occurred with the rate

TABLE 1

Manufacturing data for experimental cheese

	Lot 1115	Lot 1120
Pounds of milk	891	339
Weight of cheese made	91.25	35.5
Per cent of starter used	1.5	2.25
Incubation time at 86° F.	90 min.	150 min.
Titratable acidity of whey at curd cutting	.09%	.145%
pH of whey at cutting	6.74	6.32
pH of curd at cutting	6.75	6.28
Titratable acidity of whey at dipping	.12%	.29%
pH of whey at dipping	6.41	6.01
pH of curd at dipping	6.38	5.54
Titratable acidity of whey at milling	.45%	.8%
pH of whey at milling	5.57	5.13
pH of curd at milling	5.57	5.23
pH of curd at dressing	5.57	5.23
pH of curd after 24 hours	5.24	5.04
Per cent of water in freshly made cheese	36.9	35.5

of protein hydrolysis. Ripening of the cheese was carried on in a cold storage room, the temperature of which was controlled at 10° C. ± 1.5° C.

DISCUSSION OF EXPERIMENTAL RESULTS

Chemical Analysis

Since the data obtained by chemical analysis is comparable to the work of previous investigators, a discussion of these results will be omitted.

Analysis of Cheese by X-Ray Diffraction Methods

The manner in which x-rays have been used in this part of the study is comparable to qualitative in contrast to quantitative analysis. A series of standard x-ray diffraction patterns of the amino acids which occur in casein were made and the spacings calculated. The calculated spacings of the cheese patterns and the spacings of the mono-amino acid extract from the cheese were then correlated with the spacing values of the standard patterns of the amino acids.

As has been shown in the previous paper the diffraction pattern of casein upon x-ray analysis shows two halos at calculated spacings of 4.6 Å and 9.8 Å, which are similar to other proteins as found by Astbury (7) and others (8). The outer halo at 4.6 Å represents the perpendicular distance between the layers of polypeptide chains. Astbury has called this the "backbone" spacing. The inner halo at approximately 9.8 Å represents the length of the polypeptide side chains.

Theoretically, in terms of the dimensions of the CH_2 and CONH groups, the packing of peptide chains in the two planes should be given by 4.64 Å

and 9.68 Å, provided the amino acids of the chain are on the average of the length of valine (9). The casein values as determined, therefore, agree well with the theoretical prediction.

In order to secure the desired information about cheese ripening, diffraction patterns of the two lots, 1115 and 1120, were secured at regular intervals. Coagulation of casein by rennin and the formation of paracasein are fundamental to the process of making cheese. Accompanying this coagulation reaction marked changes occur not only in the chemical properties of the casein but in the physical as well. It becomes elastic and while warm will stretch into long strings, which shatter and break readily on cooling. However, the same x-ray diffraction patterns were obtained for casein, paracasein, and stretched paracasein. Proteins, such as keratin, and gelatin, when stretched show a marked change in structure, with a decided orientation of diffracting groups. Apparently stretching of paracasein is not a true stretching comparable to that which occurs when tension is placed on rubber or muscle fiber, but is more similar to a plastic flow.

Although the patterns of fresh cheese or paracasein are the same as the patterns for any type of casein, the x-ray pattern changes quite rapidly under certain conditions as the cheese ripens. The changes that occur may be briefly described as follows:

First, the outer halo gives way to two sharply defined rings at the inside and outside of the halo. The spacings are at approximately 4.6 Å and

TABLE 2

Calculated values for the spacings determined from the diffraction patterns, cheese 1115

Sample treatment				Ave. value for "d" spacings during period	Ave. "d" spacings for 30 other samples
1115 plain	1115 plain	1115 plain before H_2O extract	1115 after H_2O extract		
Date of exposure					
11–16–35	11–20–35	5–31–36	5–31–36		
Age of cheese in days					
1	5	196	196		
9.7	9.78	9.53	9.90	9.80	9.73
	7.58	7.60	7.61	7.62	7.55
4.6	4.55	4.55	4.57	4.58	4.60
	4.14	4.19	4.23	4.22	4.23
		3.82	3.8	3.82	3.83
					3.40
	3.01 S*	3.07 S	3.03 S	3.04	3.05
	2.89 S	2.92 S	2.91 S	2.91	2.92
	2.57 S	2.64 S	2.60 S	2.60	2.61
				2.42	2.43

* S = Strong intensity.

4.23 Å. The inner halo is retained, but a new spacing appears at 7.6 Å near the outer edge of this small inner halo. The above changes are the first to appear in the ripening process. As the period progresses other spacings are brought out in the x-ray diffraction pattern. These spacings have been calculated to be at 3.85 Å, 3.42 Å, 3.08 Å, 2.92 Å, 2.61 Å and 2.43 Å. The typical patterns of unaged and aged cheeses are illustrated by Figures 1 and 2. Figure 3 shows two patterns on the same film and illustrates clearly the sharp contrast between the diffraction rings of the unaged and the aged cheese samples.

In Tables 2 and 3 are listed the calculated values for the spacings determined from the diffraction patterns which were made throughout the ripening period of the cheese samples.

The most variable spacing in the cheese is the one which represents the length of the peptide side chain linkage. This spacing, in lot 1115, varied from 9.4 Å up to 10.1 Å. However, this value is more constant between the 1115 samples and the total variation is not as great in this lot as in lot 1120. For in this group of samples, the spacing values varied from 9.12 Å to 10.5 Å. Clark and Schaad (10) consider that the different values found for this spacing in other proteins are due to the difference in the amount of water retained by the protein. It is true that when a sample of moist cheese is ex-

TABLE 3

Calculated values for the spacings determined from the diffraction patterns, cheese 1120

Sample treatment				Ave. value for "d" spacings during period	Ave. "d" spacings for 30 other samples
1120 Plain	1120 Plain	1120 before H_2O extract	1120 after H_2O extract		
Date of exposure					
11–21–35	11–26–35	5–20–36	5–20–36		
Age of cheese in days					
1	6	181	181		
9.5	9.12	9.95	9.9	9.8	9.73
	7.68	7.65	7.65	7.66	7.55
4.61	4.65	4.7	4.62	4.63	4.60
4.3 V.F.*	4.2	4.3	4.29	4.28	4.23
			3.86	3.83	3.83
					3.40
	3.03 V.F.	3.07 S	3.07 S	3.04	3.05
	2.89 V.F.	2.94 S	2.94 S	2.90	2.92
	2.60 V.F.			2.60	2.61
					2.43

* V.F. = Very faint intensity.
S. = Strong intensity.

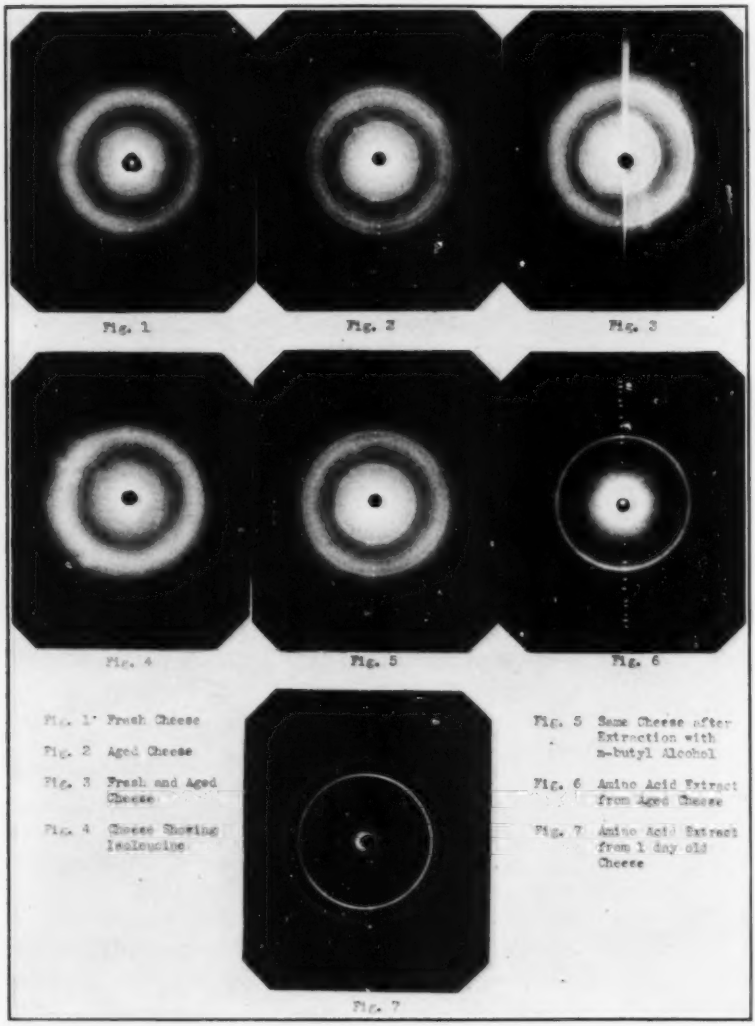
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 1 Fresh Cheese
Fig. 2 Aged Cheese
Fig. 3 Fresh and Aged Cheese
Fig. 4 Cheese Showing Isoleucine
Fig. 5 Same Cheese after Extraction with n-butyl Alcohol
Fig. 6 Amino Acid Extract from Aged Cheese
Fig. 7 Amino Acid Extract from 1 day old Cheese
Fig. 7

amined by x-rays this halo becomes indistinct and decreases in diameter, a change which indicates a larger "d" spacing.

The values for the other spacings do not exhibit the variability of the first spacing, but are surprisingly uniform. The interesting observations about the remaining spacings are the time of occurrence and the intensity of diffraction lines. The question arises as to the significance of these new spacing values in the cheese paracasein. Three possibilities might be advanced to

explain their presence. First, since cheese ripening represents an hydrolysis of protein and a liberation of amino acids, the diffraction lines may be due to amino acids. Second, the mineral constituents of the cheese may have crystallized and the characteristic pattern may be the result of the diffraction of the x-rays by the mineral salts. Third, the minerals or the amino acids may be combined with the paracasein in such a manner as to produce a crystalline pattern but still not exist as pure crystalline substances in the cheese.

If the characteristic pattern of cheese paracasein were due to free amino acids, then this pattern should be changed by the extraction of the cheese in water or hydrated n-butyl alcohol. The alcohol is a solvent for the mono-amino acids and the di-amino acids are also slightly soluble in and extracted by the n-butyl alcohol. However, the spacing values of the cheese protein are not changed by extraction of the cheese in either this solvent or water. Diffraction patterns were made of di-calcium phosphate, tri-calcium phosphate, calcium lactate and mixtures of these, but the spacing values of the cheese could not be reproduced.

On the other hand when the cheese was extracted in 10 per cent HCl, which is a solvent for both minerals and amino acids, the characteristic pattern of the cheese disappeared and the pattern reverted to one of two halos characteristic of the plain protein at the beginning of the ripening period.

In Table 4 are listed the amino acids that could possibly account for the spacing values. However, because of the similarity in structure of the amino acids the "d" spacings are approximately the same. Hence, the amino acids could not be identified specifically by these values although some weight might be attached to the fact that the spacings for isoleucine, leucine, valine, OH-proline, proline, aspartic acid and tyrosine occur most often.

Extraction of Cheese with Hydrated n-Butyl Alcohol

Dakin (6) proposed a method for the separation of amino acids into groups after the protein had been hydrolyzed by sulphuric acid. He considered that the mono-amino acids were soluble in n-butyl alcohol and could, therefore, be separated from the di-amino and di-carboxylic acids, which were supposedly insoluble in that reagent. Later investigators, notably Rose and his students, have shown that the alcohol extract does contain considerable amounts of the di-amino acids as well as proline and hydroxy prolines. However, Rose and his associates (11) were led to their discovery of the new essential amino acid, threonine, through the use of the butyl alcohol extraction method in isolating amino acids for feeding experiments.

The separation of amino acids from their mixtures is decidedly difficult because of the similarity of properties of the various amino acids. It was decided, therefore, to determine the adaptability of x-ray analysis to the identification of amino acids in the mixtures that would likely occur in the alcohol extract from the cheese.

TABLE 4

"d" spacings in Å found in cheese	"d" spacings in Å in amino acids	
7.62—(1115) 7.66—(1120)	7.61	Histidine—HCl
4.58—(1115) 4.63—(1120)	4.64 4.60 4.56 4.54	Methionine Arginine Valine Isoleucine
4.22—(1115) 4.28—(1120)	4.22 4.23 4.26 4.27	Leucine Aspartic acid Hydroxyproline Phenylalanine
3.82—(1115) 3.82—(1120)	3.80 3.84 3.84 3.86 3.87 3.87	Histidine Isoleucine Phenylalanine Aspartic acid Proline Alanine
3.42	3.4 3.4 3.4 3.4 3.45	Valine Tyrosine Proline Threonine Alanine
3.04—(1115) 3.04—(1120)	3.08 3.08 3.09	Glutamic acid Phenylalanine Histidine—HCl
2.91—(1115) 2.90—(1120)	2.90 2.93 2.95 3.00	Isoleucine Tyrosine Leucine Valine
2.60—(1115) 2.60—(1120)	2.61 2.61 2.62 2.62 2.63 2.63 2.64	Isoleucine OH—proline Proline Norleucine Valine Arginine Tyrosine
2.43—(1115)	2.40 2.41 2.41 2.41 2.41 2.46	Leucine Isoleucine Methionine Histidine—HCl Threonine Valine

Standard patterns of the amino acids occurring in casein were made, as well as patterns of physical mixtures of some of them. The spacing values for the various mixtures are illustrated in Table 5. As can be seen, when there is a mixture in equal amounts of only two amino acids (namely, leucine and isoleucine), it is relatively simple to identify them. However, when tryptophane is added to the mixture another problem arises, that of destructive interferences. There is one spacing value, 17.1 Å, that is specific for

TABLE 5

Standard spacing values for isoleucine and leucine (1 to 1) mixture			Standard spacing values for leucine, isoleucine, and tryptophane (1 to 1 to 1)		
Spacing on pattern of mixture in Å	Standard pattern value in Å		Spacing on pattern of mixture in Å	Standard pattern value in Å	
13 03	Isoleucine	13.03	17.1	Tryptophane	17.1
6.4	Isoleucine	6.52	13.02	Isoleucine	13.03
5.51	Isoleucine	5.51	6.5	Isoleucine	6.52
4.96	Leucine and Isoleucine	4.98	5.55	Isoleucine	5.51
			5.02	Leucine and Isoleucine	4.98
4.65	Leucine	4.65			
4.18	Isoleucine	4.18	4.68	Leucine	4.65
4.01	Leucine and Isoleucine	4.00 4.02	4.24	Leucine	4.22
			4.08	Leucine	4.00
3.84	Isoleucine	3.84		Isoleucine	4.02
3.47	Leucine	3.48	3.88	Isoleucine	3.88
			3.52	Leucine	3.48
3.24	Isoleucine	3.24		Isoleucine	3.50
3.16	Leucine	3.17	3.22	Isoleucine	3.24
2.95	Leucine	2.95	3.02	Isoleucine	3.00
			2.92	Leucine	2.95
2.89	Isoleucine	2.89		Isoleucine	2.89
			2.79	Leucine	2.77
2.77	Leucine	2.77		Isoleucine	2.76
2.67	Isoleucine	2.67	2.62	Isoleucine	2.60
2.60	Isoleucine	2.60	2.52	Isoleucine	2.51
			2.42	Isoleucine	2.41
2.50	Isoleucine	2.51		Tryptophane	2.45
2.40	Leucine	2.41			

tryptophane and if this spacing had not appeared, tryptophane could not have been identified as being part of the mixture. This indicates that qualitative chemical tests should also be made on mixtures so as to serve as confirmatory tests. The observation that the spacings for tryptophane were absent is significant because it held true in the unknown cheese extracts, when even one characteristic spacing value for tryptophane did not appear. However, the Hopkin's Cole test showed tryptophane to be present in all the extracts except the one from cheese one day old.

In the first part of Table 6 there is listed the values for the spacings as found in the extracted material from a cheese one day old. The spacing values are, without much doubt, due entirely to leucine and isoleucine. Apparently then, these two amino acids are liberated fairly rapidly in the hydrolysis of casein during cheese ripening. There was a negative test for tyrosine, although one would expect tyrosine to be liberated just as early as leucine and isoleucine. Evidently this was not the case. In fact, in testing all of the cheese extract samples, a much stronger test was obtained for tryptophane than for tyrosine, providing tryptophane was present at all. Womack and Rose (12) have found that leucine and isoleucine crystallize at

TABLE 6

Identification of amino acids in butyl alcohol extract from cheese

First extraction from cheese 426 age, 1 day			First extraction from cheese 2235 age, 4 months		
Value for mixture	Standard pattern value		Value for mixture	Standard pattern value	
13.9	Leucine	14.2	13.95	Leucine	14.2
			9.35	Lysine	9.05
5.25	Leucine	5.24	6.45	Tyrosine	6.55
				Isoleucine	6.53
4.55	Isoleucine	4.54			
	OH—Proline	4.52	4.75	Phenylalanine	4.75
				Leucine	4.72
				OH—Proline	4.72
			3.93	Tryptophane	3.95
4.16	Isoleucine	4.15		Leucine	4.00
			3.34	Phenylalanine	3.32
				Methionine	3.36
3.86	Isoleucine	3.84	3.23	Isoleucine	3.24
	Phenylalanine	3.84		Methionine	3.23
				Proline	3.21
3.69	Isoleucine	3.68		OH—Proline	3.22
	Histidine	3.68			
			3.10	Phenylalanine	3.08
				Histidine—HCl	3.09
2.55	Leucine	2.55		Arginine	3.10
			2.91	Isoleucine	2.91
				Tyrosine	2.93
2.34	Leucine	2.30	2.47	Isoleucine	2.41
				Methionine	2.41
				Histidine—HCl	2.41
Negative—Hopkins Cole			Positive—Hopkins Cole for Tryptophane		
Negative—Millon test for Tyrosine			Sl. positive for Tyrosine by Millon's reagent		
Negative—unoxidized sulphur test			Negative for unoxidized sulphur		

the same time when in a mixture. X-ray analysis can detect the presence of either of these when they exist in appreciable amounts in a mixture, as is shown by Table 5.

Figures 4 and 5 illustrate that leucine can be extracted from an aged cheese sample. The small inner ring on the cheese pattern of Figure 4 indicates a spacing which is specific for leucine. Upon extraction of the cheese by n-butyl alcohol, this ring disappeared. (See Figure 5.) However, the x-ray diffraction pattern of the amino acid extract shows that it is present there. (Figure 6.)

In part two of Table 6 are shown the spacing values for a cheese sample four months old. Lysine might be present, in addition to leucine, isoleucine, tryptophane, and tyrosine.

Table 7 lists the values for the extract from a cheese sample approximately eighteen months old. The presence of leucine and isoleucine are clearly illustrated. Tyrosine and tryptophane were shown to be present by the characteristic color tests for amino acids that were applied to the crystalline material.

TABLE 7

Identification of amino acids in butyl alcohol extract from cheese

First extraction from cheese 12274 age, 15 months			First extraction from commercial cheese age, approximately 18 months		
Value for mixture	Standard pattern value		Value for mixture	Standard pattern value	
13.9	Leucine	14.2	14.3	Leucine	14.2
7.31	Aspartic acid	7.42	7.31	Aspartic acid	7.42
5.21	Leucine	5.24	4.99	Isoleucine	4.99
				Leucine	4.98
4.60	Isoleucine	4.56		Tyrosine	4.90
	Arginine	4.60			
			4.56	Isoleucine	4.54
4.00	Leucine	4.00		Proline	4.53
	Isoleucine	4.02		OH—Proline	4.52
	Phenylalanine	3.99			
			4.07	Aspartic acid	4.06
3.65	Isoleucine	3.68		Isoleucine	4.03
	Serine	3.66		Tyrosine	4.09
	Phenylalanine	3.64		Threonine	4.09
	OH—Proline	3.64			
			3.58	Tyrosine	3.59
3.25	Isoleucine	3.24			
	Proline	3.21	3.23	Isoleucine	3.24
	OH—Proline	3.22		OH—Proline	3.22
				Methionine	3.22
2.97	Leucine	2.96			
	Arginine	2.97	3.12	Leucine	3.15
	Lysine	2.95		Aspartic acid	3.13
				Lysine	3.13
2.82	Leucine	2.82			
	Threonine	2.82	2.80	Leucine	2.82
				Proline	2.80
2.40	Leucine	2.40			
	Isoleucine	2.41			
	Arginine	2.40			
	Threonine	2.40			
Positive—Hopkins Cole test Sl. positive—Millon's test			Positive—Hopkins Cole test Positive—Millon's test		

Figures 6 and 7 show the x-ray diffraction patterns of the amino acid extracts from two different samples of cheese. As one can observe, there is a difference in the patterns, indicating that the crystalline extracts were not identical in composition in all respects. The calculations of the spacing values listed in the Tables 6 and 7 also show that this is true.

A Long Interplanar Spacing in Cheese

Clark and Schaad (10) have shown that certain proteins; namely, nerve tissue and intestinal wall collagen, contain certain long spacings that can be isolated by a special technique. Spacings varying from 70 Å up to 400 Å have been found in intestinal wall collagen when using a vacuum camera and a distance of 20 cm. from specimen to film. The long spacing which was found in cheese, but which was absent from casein, was calculated to be

39.7 Å. However, the nonappearance of the long spacing interference in pure casein is still unaccounted for.

Svedberg (13) calculated the radius of the casein particle, assuming that it was spherical, to be 41.7 Å to 59.94 Å. The two values, 41.7 Å for casein and 39.7 Å for cheese, are of the same order of magnitude. Therefore, it is possible that this spacing represents the radius of the casein particle, or the maximum length of the side chain spacing from the main chain. If this be true, the size of the casein particle which has been arrived at by another method agrees well with that determined by Svedberg.

SUMMARY

An attempt has been made to follow the changes which occur during the ripening of cheddar cheese by x-ray diffraction analysis.

The diffraction pattern of the cheese protein after aging is markedly different from the pattern of the fresh cheese. The pattern appears to be due to crystalline material, although the identification of the material has not been made as yet.

Standard patterns were made and "d" spacing values were calculated for the amino acids occurring in casein in order to determine whether the characteristic pattern was due to an amino acid of low solubility.

By the extraction of the aged cheese in hydrated n-butyl alcohol, amino acids were extracted from the cheese and identified. Leucine and isoleucine were obtained from cheese 24 hours after being made. Tyrosine, and probably tryptophane were also identified in the extract obtained from ripened cheese. A long interplanar spacing of 39.7 Å was obtained from cheese protein. This spacing represents the length of a side chain from the main chain and corresponds closely with the radius of the casein particle which Svedberg determined to be 41.7 Å to 59.9 Å

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JUDGING SWEET CREAM

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The more general application of scientific methods in the production, manufacture and merchandising of dairy products during the last quarter century has very markedly improved the quality of the goods which the industry offers to the consumer. Whether one contemplates large scale production such as is realized in the thrice-daily milking of 1600 cows on one large eastern farm, or a city pasteurizing plant processing 400,000 pounds or more of milk daily, or the nation-wide marketing methods of the larger dairy manufacturers, one is everywhere impressed by the emphasis laid on the fine, healthful quality of the product. This growing stress on quality is making the consumer more discriminating in the selection of brands or sources of supply and would seem to require that the industry reexamine carefully the methods of measuring quality which are currently employed for this purpose.

In the past little attention has been given to the evaluation of the quality of sweet fluid cream offered to the public for use in coffee, with fruits and cereals, or as a whipped cream. It is true that the U. S. Department of Agriculture some years ago adopted, with the approval of the American Dairy Science Association, a score card for the alternative judging of milk or cream. (1) This score card allotted 40 points to physical characteristics, divided into 25 points for flavor, 10 for sediment and 5 for package, and 60 points to laboratory-tested qualities, of which 45 depended on total plate counts of microorganisms and 15 on titratable acidity or, alternatively, the temperature of "street" samples.

Regardless of opinion as to whether such a weighting of the several factors is justifiable or desirable in judging fluid milk as marketed today, there can be little doubt that sweet fluid cream should be rated on a different basis. In the first place, the volume of milk now being sold in the form of sweet cream is sufficient to warrant separate consideration for this product, and, in the second place, the qualities which determine the marketability of cream differ considerably from those which determine the marketability of milk.

Statistics compiled by the Federal government in several of the large eastern markets (2) show that at least 40 per cent of the total fluid milk volume consumed in these markets is distributed in the form of fluid cream. This relationship may not hold exactly in all sections of the United States, but is probably applicable to most urban centers where the bulk of sweet cream sales occurs.

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It is evident that sweet cream occupies, both from the dollar value and from the volume of milk required for its manufacture a very high place in the economics of fluid milk distribution.

The question arises as to what characterizes high quality in fluid cream. Certainly flavor is of prime importance, since a large proportion of the fluid cream sold is consumed on fruits, cereals, cake, etc., which do not mask the flavor of the cream itself. Off-flavors in cream are a primary source of customer complaints. For this reason the distributor of fluid cream is very conscious of the necessity of excellence of flavor. It would seem reasonable in scoring cream that flavor should be given as much consideration as in scoring butter, where forty-five points out of 100 are allotted to flavor.

"Pourability" or consistency, or viscosity, is another factor of prime importance in the eyes of the customer. Being aware of the general association of increased body with higher fat content, the housewife suspects a thin-pouring cream as being low in butterfat and is likely to enter a complaint with her distributor.

Whipping quality is probably more troublesome today to the milk distributor than any other characteristic of fluid cream. Many resort to the costly expedient of increasing the fat content to satisfy their customers as to whipping quality. Others add "viscogen," lecithin or skim milk solids, practices which are illegal in many areas and which are eschewed by leaders in the industry as partaking of adulteration.

Cream "plug," giving rise to oiliness in coffee, is a frequent defect of fluid cream, while the separation of a serum layer in bottled cream is a very common occurrence in the lower fat ranges. Sediment, showing lack of cleanliness in production and handling, must always be considered in judging quality. The appearance of the container is a factor which is being emphasized in all food merchandising today and should be accorded some weight in any rating of quality of a packaged food.

The present score card for milk and cream allots 45 points to total bacterial counts, giving a cream with a plate count of 100,000 organisms per ml. a zero score in this bracket. This emphasis on bacterial content seems entirely out of proportion to its importance today. Under modern conditions of city milk regulation and inspection the general condition of fluid cream from a bacteriological standpoint is quite satisfactory. The public is adequately protected from pathogenic organisms by pasteurization requirements, and the subsequent protection of the product against temperature change, so that total counts are of importance mainly because of the effect of putrefactive and acid types of organisms on flavor and keeping quality. If samples are judged twenty-four hours after processing, the effect of objectionable high counts will probably be manifested in off-flavors. For these reasons we believe that 15 points gives sufficient weight to bac-

terial counts, penalizing a sample one point for each 10,000 per ml. count above 10,000. By this means any cream containing in excess of 160,000 bacteria per ml. would be penalized 15 points.

Acidity is of importance as it affects keeping quality and flavor. Prevailing conditions of producing and processing sweet cream result in low acidities as well as low bacterial counts at time of packaging. Since subsequent carelessness in handling may lead to bacterial growth and developed acidity, a small penalty for acidity increase would appear to serve for

TABLE I

Cream Score Card

Sample No. ———— Butterfat Content ———— Date ————

Judge after 24 hrs. aging Enter observed data—Underline defects			Perfect score	Points deduct.	Score allow.
FLAVOR:	Exc. (34–40)	No definite criticism	40	———	
	Good (28–33)	Very slight feed, sl. cooked		———	
	Fair (23–27)	Cooked, feed, sl. unclean or metall.		———	
	Poor (13–22)	Metallic, str. feed, unclean, sl. acid, sl. rancid or tallowy		———	
	Bad (0–12)	Sour, foreign, rancid, tallowy, putrid		———	———
BODY:	*Light Cream Score*	*WHIPPING*	20		
———sec.	Exc. (92 to 100%)	18–20 Sec. to whip ———			
———sec.	Fair (72–92%)	13–17 4 points for Exc.			
———sec.	Poor (48 to 72%)	7–12 Cm. Penetr. ———	Body	———	
	Heavy Cream Score	2 points for Exc.			
———sec.	Exc. (as above)	9–10 Volume Incr. ———%			
———sec.	Fair (" ")	6– 8 2 points for Exc.			
———sec.	Poor (" ")	2– 5 Ml. Leakage ———	Whip.	———	
Refer to tables for Excellent standards		2 points for Exc.	Total	(20 max)	———
N.B. If whipping tests not made, score *Heavy Cream* Body same scale as light.					
BACTERIA COUNT:	10,000 or less considered perfect For higher counts deduct 1 point per 10,000 additional		15		
———per ml.				———	———
CREAM PLUG:	Deduct: 0–1 Sl., soft, foamy plug 1–2 Distinct soft plug 2–3 Buttery plug 4–5 Leathery plug		5	———	———
SERUM SEPARATION:	Deduct 1 point for each 1/16" serum		5	———	———
ACIDITY:	Deduct 1.0 for each 0.1% acid above normal acidity for cream of specified fat content		5	———	———
SEDIMENT:	Penalize in proportion to specks or dirt		5	———	———
PACKAGE:	Dirty bottle—deduct 0.5–1.0 Bottle etched or chipped—deduct 0.25–1.0 Slack fill—deduct 1.0 for each ¼" short Unprotected lip—deduct up to 1.0 point Poor cap condition—deduct up to 1.0		5	———	———
COMMENTS:			TOTAL SCORE————		
			Scorer ————		

quality maintenance. Actual souring would be severely injurious to good flavor and would bring a correspondingly heavy penalty in that part of the score.

With these ideas in mind it appeared desirable to propose a new method for the evaluation of the quality of fluid cream, one which would reflect more accurately the various factors entering into its determination and one which might be better adapted to conditions in the industry today. We believe that this subject merits thoughtful consideration by dairy science workers, both in the academic and in the industrial fields. In the following scheme (Table I) which has had a year's practical trial in a number of fluid milk plants with favorable endorsement, 40 points are allotted to flavor, 20 points to body or body and whipping quality, 15 points to bacterial counts, 5 points each to "plug," serum separation, acidity, sediment and package.

COMMENTS ON SCORE CARD

Quart samples of cream are required to complete all determinations required by the score card. All measurements should be made on creams 24 hours after bottling.

The off-flavors referred to are so well-recognized and defined as to require no elaboration here. Any particular criticisms may be indicated by underlining the appropriate terms. Body and whipping quality present more of a problem in evaluation. Twenty points are suggested for this group, all of which are allotted to body for creams of lower fat content, but are divided between the two for those classified as "whipping creams." Hening (3) has discussed the measurement of the viscosity of cream by means of the MacMichael viscometer and the Borden Flow Meter. It is proposed to use these instruments alternatively, scoring this quality according to the reading obtained. In Table II there is suggested a range of

TABLE II

Viscosity of fluid cream at 15.5° C. (60° F.)

% B.F.	Body flow time (seconds)		Viscosity (centipoises)		% B.F.	Body flow time (seconds)		Viscosity (centipoises)	
	Exc.	Poor	Exc.	Poor		Exc.	Poor	Exc.	Poor
18	38 –	26	2 –	1.75	32	75 –	42	18 –	6
20	40 –	28	7 –	2.0	34	90 –	48	22 –	12
22	44 –	30	8 –	2.25	36	120 –	60	29 –	14
24	48 –	32	11 –	2.5	38	170 –	85	38 –	22
26	52 –	34	12 –	2.5	40	230 –	115	47 –	25
28	58 –	36	14 –	3.	42	290 –	145	60 –	34
30	65 –	38	15 –	4.	44	360 –	180	65 –	39

expected values which experience indicates to be reasonable for pasteurized cream aged 24 hours in the original package.

The numerical score to be assigned to a cream of a given fat content is determined by the percentage of the "Excellent" value obtained for the particular sample, as indicated in the score card.

WHIPPING QUALITY

If whipping creams are to be rated for whipping quality, it is essential to employ a standard method which is simple, easily reproducible and employs inexpensive equipment. Several procedures have been described by investigators (4, 5, 6, 7, 8, 9, 10). It is believed the following one well meets the requirements laid down:

Equipment

1. "Duplex" whipper and bowl.*
2. 250 and 100 ml. graduates.
3. 600 ml. beakers.
4. 40 gm. Penetrometer (see Figure 1).
5. 90 mm. 60° glass funnels.
6. 1″ wire screen discs (20 mesh per inch)
7. Thermometer, grease pencil, spatula, stop watch.

Method

While the score card should normally be applied to samples 24 hours after pasteurization, it may happen that the bottle of cream has warmed up somewhat before examination. For this reason it is desirable to make certain that the sample has been held below 7° C. (44.6° F.) for at least two hours prior to testing. Cool whipper and bowl to same temperature before making test.

1. Measure 200 ml. of cream into bowl. Adjust temperature to 7° C. With uniform speed of whipper (120 R. P. M.), record *whipping time* in seconds to whip cream to maximum stiffness. Avoid over-whipping.

2. Transfer whipped cream to a 600 ml. beaker, gently tap bottom of beaker on palm of hand to pack and level the whipped cream, mark the surface level with grease pencil for making *volume determinations* later.

3. Determine *stiffness of whip* with penetrometer. Tighten glass sleeve of instrument in burette clamp at a convenient height. Raise penetrometer with fingers so beaker may be set beneath it. Lower the stem until disc just rests on surface of the whipped cream. Release fingers after noting reading on stem opposite upper edge of sleeve. Record penetration in cms./min.

4. Transfer whipped cream to glass funnel fitted with wire disc as a support for cream. Place graduated cylinder under funnel to collect leakage. Expose samples for 2 hours at room temperature (20–22° C.) Record *leakage* in milliliters for 2 hour period.

5. After removing whipped cream from beaker, fill to whipped cream level with water and measure for *volume*. Record the percentage increase in volume.

* Manufactured by V. V. Vale Corporation, Oak Park, Illinois.

Ten points are allotted to whipping quality, divided between stiffness, volume increase, leakage and time to whip. In Table III is suggested a tentative criterion for evaluation of these factors.

TABLE III

Whipping quality of cream

	Excellent	Fair	Poor
Time to whip	Under 40 seconds	40–90 seconds	Over 90 secs.
Stiffness	No penetration	0–2.0 cm./min.	Instant penetration
Volume increase	90–110%	70–90%	70% or less
Leakage	< 4 ml.	4–14 ml.	14–20 ml.

Blank spaces are provided on the score card for recording the actual measurements in determining body and whipping quality.

BACTERIA COUNT

Total plate counts are determined by the A. P. H. A. standard method for milk. If less than 10,000 per ml. the sample receives a full score of 15. One point is deducted for each ten thousand or fraction thereof above ten thousand per ml.

SERUM AND PLUG

Serum separation is easily recognized by the distinct bluish layer in the bottom of the bottle, which frequently occurs following storage. "Plug" is best observed through removal of the bottle cap. It is objectionable to the consumer because it hinders pouring and causes oiliness on the surface of hot coffee. The deductions proposed in the score card are self-explanatory.

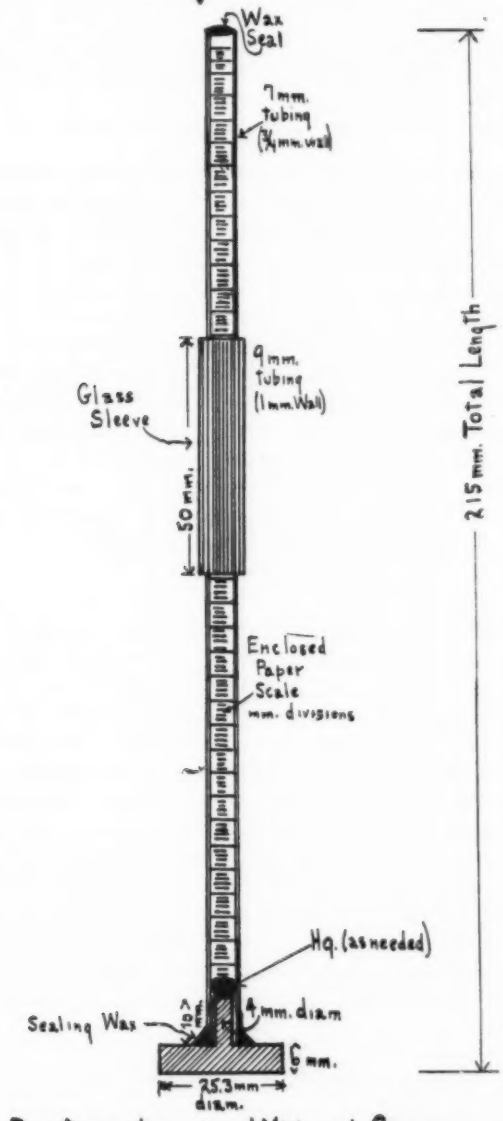
ACIDITY

The acidity should be determined by titration of a 9 cc. portion of the cream diluted with 9 cc. of distilled water, using N/10 alkali and 3 drops of phenolphthalein indicator. Since the acidity of cream depends upon the serum present, it will vary inversely with the fat content of the cream. Hence, limits for permissible acidity are determined by calculating the observed titratable acidity value to the basis of the serum alone. A reading of 0.11 per cent in 40 per cent cream is equivalent to 0.18 per cent in skim milk and represents the maximum permissible for a perfect score. One of over 0.14 per cent in 40 per cent cream, equivalent to 0.23 per cent in the serum portion, would be scored zero. Corresponding limits for 20 per cent fat cream would be 0.145 per cent and 0.185 per cent acidity. For intermediate fat contents similarly calculated limits should be used.

SEDIMENT AND PACKAGE

Deductions for sediment are based upon the appearance of filter discs obtained by filtering pint samples of cream. A method for preparing such

Fig. 1



Penetrometer for Whipped Cream

discs has been proposed (11) which consists of diluting one pint of cream with an equal volume of filtered hot water, and filtering it through one of the standard milk sediment testers at a temperature of about 50° C. Deductions for sediment are made in proportion to the dirt on the disc.

The defects in package are the same as those now recognized in scoring milk and cream.

PLANT APPLICATION

The proposed method for determining the quality of sweet cream has received practical trial in the laboratories of a number of plants. Some have used the score card as a daily routine procedure, particularly for light cream. Others have employed it in periodical surveys of samples distributed in given local areas. The results of the examination of several hundred creams of different fat contents have been recorded. The scores ranged from a maximum of 98 to a minimum of 48, indicating the potential value of such a numerical expression of measurable characteristics as a yardstick by which high quality can be maintained in day-to-day production. Comments made by individual workers indicate their appreciation of the value of such a written record. The score card in its present form appears to offer a convenient, workable tool for routine laboratory control purposes as well as an excellent technique for comparing the quality of a group of cream samples as to their consumer appeal.

CONCLUSIONS

It is the belief of the authors that present-day quality in sweet cream as distributed in urban centers merits careful consideration of a number of characteristics not recognized in the score card now used interchangeably for milk and cream. At the same time, modern practice makes a different weighting of individual qualities appear desirable. A separate score card for cream is suggested, with proposed methods for judging the respective characteristics, and details of application are given. The suggested scheme has received practical trial in a number of commercial control laboratories, where it has proven workable and convenient, reflecting in an exact manner the differences which are distinguishable in a general way between samples of cream. The official adoption of such a score card, or some suitable modification of the same, would provide both the academic and the industrial investigator with a recognized standard method for evaluating cream quality as it is offered to the public under modern marketing conditions.

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FACTORS AFFECTING THE ACTIVITY AND HEAT RESISTANCE OF SWISS CHEESE STARTER CULTURES. I. INFLUENCE OF TIME AND TEMPERATURE OF INCUBATION[1]

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Frazier and coworkers (7), in their studies on the bacteriology of Swiss cheese, found that cultures of *Lactobacillus helveticus* 39a, incubated at 38° to 39° C., developed more actively during the manufacture of Swiss cheese than did those incubated at 35° to 37.5° C. Unpublished results indicated that when *L. helveticus* had been grown in milk at 30° C. for 24 hours, it showed definitely less activity in the cheese on the press than when it had been grown at 37° C. for 12 hours. Despite the fact that cultures grown at the two temperatures produced about the same amount of titratable acid at the end of their respective incubation periods and presumably were of approximately the same maturity, the culture grown at the higher temperature had the greater ability to withstand heating.

During the manufacture of Swiss cheese, the temperature of the curd is raised in a period of about 30 minutes from the average setting temperature of 33° C. to an average cooking temperature of 53° C. The curd is held at or slightly below this temperature for about 30 minutes, after which it is removed from the kettle and placed in the press. The temperature falls very slowly during the next 24 hours, particularly in the center of the cheese. It is obvious then than any organisms which develop in the early hours on the press must be both heat resistant and capable of developing readily at temperatures near their maximum.

A study was made to determine the influence of time and temperature of incubation on heat resistance and activity following the heating of certain commonly used Swiss cheese starter organisms.

The literature on influence of time and temperature of incubation on heat resistance of bacteria has been reviewed briefly by Elliker (4) and by Elliker and Frazier (5).

Numerous investigations have demonstrated that age of bacterial cells markedly influences their resistance to heat. Frazier *et al.* (7) have shown the importance of age of the cultures. The temperature inducing greatest heat resistance of vegetative cells has been found to vary with the type of organism. For example, Claydon (2) and Anderson and Meanwell (1) reported that *Streptococcus lactis* and a thermoduric streptococcus, respectively, showed greatest heat resistance when grown at temperatures below the

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optimum for growth. Dorner and Thöni (3) demonstrated that there was little difference in heat resistance of cultures of *Bacterium acidi propionici* grown at 22° and at 30° C. Unpublished results obtained at the Washington laboratories of the Bureau of Dairy Industry indicated that propionic acid bacteria grown at temperatures near their optimum were more resistant to heat than those grown at lower temperatures. Results reported by Frazier and coworkers (7), and by Elliker and Frazier (5) demonstrated that with certain organisms incubation at temperatures near or above the optimum for growth might result in greater heat resistance and activity following heating than would result from incubation at a lower temperature.

EXPERIMENTAL

In this investigation on the heat resistance of non-spore forming, thermoduric bacteria, *Lactobacillus helveticus* 39aW and *Streptococcus thermophilus* C-3, the organisms used by Frazier *et al.* (7), were used. In part of the work several other species of *Lactobacillus* and *Streptococcus* were employed.

The usual method of measuring heat resistance of a culture is to determine the number of organisms present both before and after heat treatment by plate counts and from these calculate the percentage surviving. This method is not adapted for the study of the heat resistance of *L. helveticus* and *Str. thermophilus* because of the tendency of these organisms to form long chains, particularly in young cultures and at high temperatures. For this reason activity following heating of the culture was followed by direct microscopic counts, and by acid titrations made before and at varying intervals following the heat treatment. This method measures the ability of the culture to grow and ferment after treatment. The main disadvantage is that it does not determine the percentage survival of cells. In these studies, a measure of the activity following heating was used more than the plate count method.

The cultures were carried in tubes of sterile reconstituted skim milk, always prepared from the same lot of skim milk powder. Unless otherwise indicated, one per cent of inoculum was used. Stock cultures were incubated at 37° C. for 16 hours after which they were kept at 10° C. until the next transfer one week later. Determination of heat resistance or of activity after heating of a culture was carried out on an inoculating culture which was prepared by transfer of one per cent of inoculum from the most recent stock or mother culture to 100 cc. of milk. Unless otherwise indicated the incubation was the same as that of the stock or mother culture. Following incubation, 0.25 per cent of culture was transferred from the inoculating culture to a liter flask containing 450 cc. of freshly steamed and cooled sterile reconstituted skim milk. The temperature of the milk in the flasks was raised in a period of about five minutes to the temperature of

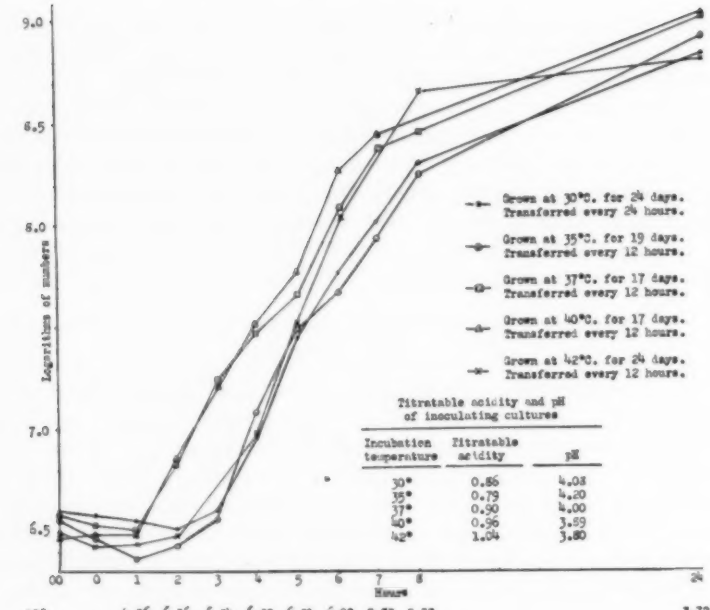
heat treatment, usually 60° C., maintained for 30 minutes and then lowered to the temperature of subsequent incubation. It was believed that this treatment reasonably simulated the exposure which starter cultures might receive in the manufacture of Swiss cheese. Incubation following heat exposure was carried out in thermostatically controlled water baths. The rate of growth following the heat treatment of the cultures was determined by direct microscopic counts of living cells according to the method of Frazier and Boyer (6), and also by determinations of pH and titratable acidity. Samples were removed for these determinations before and at definite intervals following the heat treatment.

Influence of temperature of incubation on activity following heat treatment

In this experiment cultures were carried at 30°, with transfers every 24 hours, and at 35, 37, 40 and 42° C. with transfers every 12 hours. These are incubation periods commonly used in carrying starter cultures in actual practice. It was believed that growth at temperatures in this range might yield some knowledge of the effect of incubation temperature on heat resistance of the organisms, and that consequently the studies might be limited to the influence of two or three different temperatures. Such a procedure would then allow more detailed and better controlled experiments than would be possible if a greater number of temperatures were used. It was realized that the incubation temperatures and times used resulted in cultures whose maturity, as indicated by titratable acidity and pH of inoculating cultures, varied to some extent. Nevertheless, results obtained by the carefully controlled methods used should give some indication of the effect of temperature and should suggest whatever subsequent modifications of methods might be necessary.

Figures 1 and 2 show the relative activity following heat treatment of cultures carried at the various temperatures. Growth curves constructed from direct counts indicate the rate of growth at 37° C. following heat treatment. Also included are titratable acidities and pH values for the first eight hours and the 24th hour, as well as acidities of the inoculating cultures. Samples removed from the flasks of milk before heat treatment are designated in the figures as 00 hour samples; those removed immediately after heat treatment and cooling to 37° C. are designated as 0 hour samples.

The activity of the inoculating culture following heat treatment could be predicted from its titratable acidity at the time it was used. Repeatedly inoculating cultures with low titratable acidities were found to be inactive after the heat treatment. The titratable acidity produced in an inoculating culture within a given time at a given temperature, therefore, indicates how active that culture will be following subsequent heat treatment. Frazier *et al.* (7) have emphasized the importance of a certain titratable acidity in


9.0
8.5
8.0
7.0
6.5
Logarithm of numbers
Grown at 30°C. for 24 days. Transferred every 24 hours.
Grown at 35°C. for 19 days. Transferred every 12 hours.
Grown at 37°C. for 17 days. Transferred every 12 hours.
Grown at 40°C. for 17 days. Transferred every 12 hours.
Grown at 42°C. for 24 days. Transferred every 12 hours.
Titratable acidity and pH of inoculating cultures
Incubation temperature | Titratable acidity | pH
30° 0.86 4.08
35° 0.79 4.20
37° 0.90 4.00
40° 0.96 3.89
42° 1.04 3.80
00 0 1 2 3 4 5 6 7 8 24
Hours

pH	30°	6.36	6.36	6.34	6.29	6.21	6.09	5.83	5.53		3.72
	35°	6.34	6.33	6.31	6.28	6.21	6.07	5.86	5.58		3.74
	37°	6.36	6.34	6.30	6.27	6.18	6.01	5.78	5.39		3.72
	40°	6.36	6.34	6.30	6.25	6.16	5.93	5.65	5.20		3.68
	42°	6.36	6.36	6.32	6.26	6.15	6.00	5.73	5.41		3.74
Titratable acid	30°	0.15	0.15	0.15	0.16	0.17	0.19	0.22	0.28		1.20
	35°	0.15	0.15	0.15	0.16	0.17	0.19	0.22	0.26		1.14
	37°	0.15	0.15	0.15	0.16	0.18	0.21	0.25	0.31		1.19
	40°	0.15	0.15	0.16	0.18	0.19	0.22	0.26	0.34		1.20
	42°	0.14	0.15	0.15	0.16	0.17	0.20	0.22	0.28		1.21

FIG. 1. Growth curves and acid production at 37° C. of cultures of *Lactobacillus helveticus* grown at different temperatures and heat shocked at 60° C. for thirty minutes.

bulk starter cultures within a given incubation time at a given incubation temperature if the starter organisms are to be active during the manufacture of Swiss cheese.

Examination of Figure 1 leaves little doubt that the 37° and 40° cultures were superior to the others. The pH and titratable acidity of the respective inoculating cultures indicate that those grown at 30° and 35° had not reached as high a stage of maturity as had the 37°, 40° and 42° C. cultures. The activity of all cultures except the 42° was directly proportional to the titratable acidities of the inoculating cultures. This indicates that the maturity of the cells in the inoculating culture may influence their subsequent heat resistance. The counts were higher in those cultures grown at the lower temperatures, therefore the original number of organisms present before heat treatment did not greatly influence the results.

TABLE 1

Influence of incubation temperature of mother cultures on the activity of Lactobacillus helveticus and Streptococcus thermophilus at 37° C. (98.6° F.) following heat shocking

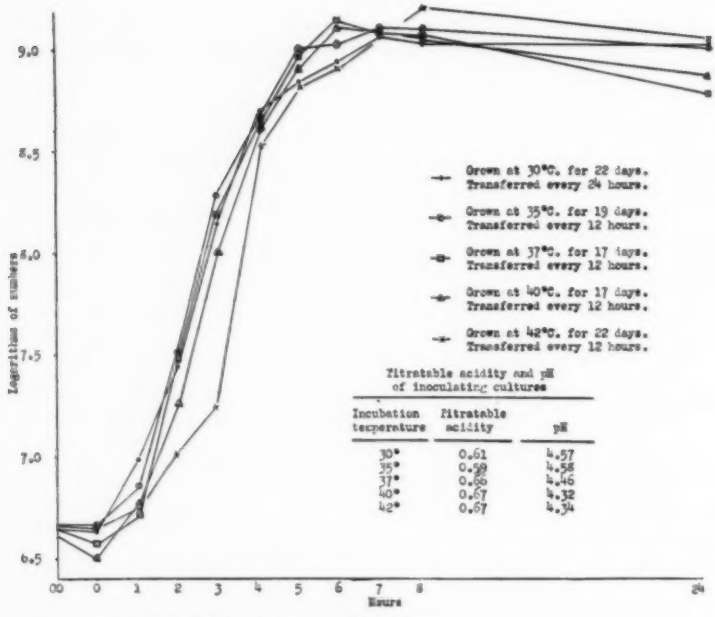
Culture	Incubation time and temperature	Number of transfers at respective temperatures	Titratable acid of inoculating culture	Time and temperature of heat shocking	Time of incubation following heat shocking	Drop in pH
			Per cent		*Hours*	
L. helveticus	12 hrs. 37°	1	0.87	30′ 65°	13	.36
	12 hrs. 40°	1	0.99	30′ 65°	13	.29
L. helveticus	12 hrs. 37°	34	0.90	30′ 60°	6	.35
	12 hrs. 40°	34	0.96	30′ 60°	6	.43
L. helveticus	12 hrs. 37°	58	0.92	30′ 62.5°	8	.86
	12 hrs. 40°	58	1.11	30′ 62.5°	8	.98
Str. thermophilus	12 hrs. 37°	1	0.63	30′ 65°	5	.22
	12 hrs. 40°	1	0.67	30′ 65°	5	.00
Str. thermophilus	12 hrs. 37°	4	0.64	30′ 70°	12	.36
	12 hrs. 40°	10	0.68	30′ 70°	12	.04
Str. thermophilus	12 hrs. 37°	16	0.64	30′ 60°	5	1.00
	12 hrs. 40°	16	0.67	30′ 60°	5	.86
Str. thermophilus	12 hrs. 37°	24	0.66	30′ 65°	5	.16
	12 hrs. 40°	24	0.65	30′ 65°	5	.01

Counts and pH values following heating of cultures of *Str. thermophilus*, shown in Fig. 2, indicated that the 30° and 35° cultures initiated growth first, closely followed by the 37° C. culture. The acidities of the 30° and 35°, and possibly the 37° cultures, are lower than acidities of cultures grown at the other temperatures.

The differences in rate of acid formation at 37° C. following heat treatment were slight, yet they were significant. This fact was emphasized in later experiments in which cultures were incubated at temperatures near their maximum following heat treatment.

Effect of incubation of L. helveticus and Str. thermophilus at 37° and 40°, with transfers every 12 hours, on their activity following heat treatment

The results shown in Figures 1 and 2 demonstrate that growth at 37° and 40° C. appeared to increase slightly the heat resistance of *L. helveticus* and that there was a slight superiority of the 40° over the 37° culture. From the results of Frazier *et al.* (7) and those obtained in this work, it seemed logical to assume that if the effect of temperature on heat resistance of


Logarithm of numbers
9.0
8.5
8.0
7.5
7.0
6.5
Hours
Grown at 30°C. for 22 days. Transferred every 24 hours.
Grown at 35°C. for 19 days. Transferred every 12 hours.
Grown at 37°C. for 17 days. Transferred every 12 hours.
Grown at 40°C. for 17 days. Transferred every 12 hours.
Grown at 42°C. for 22 days. Transferred every 12 hours.

Titratable acidity and pH of inoculating cultures

Incubation temperature	Titratable acidity	pH
30°	0.61	4.57
35°	0.59	4.58
37°	0.66	4.46
40°	0.67	4.32
42°	0.67	4.34

		0	1	2	3	4	5	6	7	8	24
pH	30°	6.36	6.33	6.14	5.87	5.31	5.06	4.92	4.83		4.12
	35°	6.34	6.33	6.14	5.89	5.31	5.06	4.92	4.83		4.21
	37°	6.34	6.32	6.16	5.89	5.30	5.10	4.95	4.82		
	40°	6.34	6.33	6.21	5.96	5.38	5.13	4.97	4.82		
	42°	6.36	6.35	6.25	5.99	5.45	5.16	4.97	4.88		4.12
Titratable acid	30°	0.15	0.15	0.19	0.26	0.35	0.40	0.44	0.48		0.76
	35°	0.15	0.16	0.18	0.25	0.35	0.40	0.45	0.47		0.77
	37°	0.15	0.16	0.21	0.27	0.37	0.41	0.47	0.48		0.78
	40°	0.15	0.15	0.19	0.25	0.34	0.41	0.45	0.48		0.77
	42°	0.15	0.15	0.17	0.22	0.32	0.39	0.43	0.46		0.77

FIG. 2. Growth curves and acid production at 37° C. of cultures of *Streptococcus thermophilus* grown at different temperatures and heat shocked at 60° C. for thirty minutes.

these organisms was significant, carefully controlled, parallel studies on cultures carried at 37° and 40° should bring out differences between them.

Because the higher incubation temperatures, such as 40° C. and above, apparently were unfavorable for *Str. thermophilus*, growth of this organism at 37° and 40° might result in an effect opposite to that obtained with *L. helveticus*. Accordingly, these two organisms were carried for a varying number of transfers at 37° and 40° with transfer every 12 hours. In certain cases cultures were heat shocked after only one transfer, in other cases after numerous transfers. The temperature of heat shocking was altered in certain experiments in order to emphasize differences not brought out by a less severe heat exposure. Because the presentation of all the pH values and counts would require a large number of tables, the change in pH

during a definite period following heat treatment is tabulated rather then the pH values, and counts for every individual hourly determination. It will be observed that in the tables the intervals between time of heat shocking and time of determination of acidity are varied in proportion to rate of acid formation following heat treatment.

The results shown in Table 1 indicate that following one transfer at the two temperatures, the 37° *L. helveticus* culture was better able to withstand the heat treatment, but after 34 transfers, the 40° appeared superior to the 37° culture. After 58 transfers, the 40° C. culture was still superior. It is apparent, then, that some change had taken place in the comparative resistance of the two cultures during continuous transfer at the two temperatures. *Str. thermophilus* exhibited a behavior altogether different from that of *L. helveticus.* Results in Table 1 indicate that after one transfer, the 37° *Str. thermophilus* culture was decidedly the more resistant to heat exposure. In the five hours following heat shocking the pH of the 37° culture dropped 0.22 while no measurable acid was formed in the 40° culture. After numerous transfers, similar results were obtained. Heat shocking at 65° rather than 60° emphasized this difference. After the twenty-fourth transfer, the 37° culture lowered the pH a total of 0.16 during the first five hours after heat shocking while the 40° culture lowered it only 0.01.

It was demonstrated further that the greater heat resistance of the 40° *L. helveticus* culture was not eliminated by one transfer at a temperature of 37° C. After the mother cultures had been carried at 37° and 40° C., respectively, for 60 days, one inoculating culture was inoculated from the 37° mother culture and incubated at 37° C. for 12 hours. A second inoculating culture was inoculated from the 40° mother culture and incubated at 40° for 12 hours. A third was inoculated from the 40° mother culture and incubated at 37° C. for 12 hours. The 40° culture grown continuously at 40° was more resistant than the same 40° culture grown for one transfer at 37°. However, both of these cultures were more resistant than the 37° culture grown continuously at 37° C.

Effect of continuous transfer of mother cultures at 37° and 40° C. on their activity at temperatures near their maximum after heat shocking

In previous experiments a temperature of 37° C. was employed following heat shocking to compare ability of cultures to develop following exposure to heat. In the manufacture of Swiss cheese any organisms which are to develop in the earlier stages (the time when cultures of *Str. thermophilus* and lactobacilli should be active) must be able to initiate growth at the high temperature of the curd at the time it is placed in the press and must continue to grow at the high temperatures which are maintained while the

cheese is on the press. Results above show definite but not pronounced differences between *L. helveticus* grown at 37° and at 40° C. Nevertheless field results and those of Frazier *et al.* (7) demonstrated that a slightly higher incubation temperature of bulk starters resulted in greater activity of organisms when the curd was on the press. It was considered possible that the apparent superiority of the 40° over the 37° C. culture might be due to the fact that the change in a culture produced by continued growth at the higher incubation temperature was the ability to grow well subsequently at higher temperatures (*e.g.*, near the maximum temperature) rather than any effect on survival and on activity of cells at 37° after heating. For this reason in this and in certain of the following experiments, after the cultures were heat shocked at 60° C. for 30 minutes, they were incubated at temperatures near their maximum. Counts and pH determinations were made at definite intervals to compare development at the higher tempera-

TABLE 2

Influence of incubation temperature of mother cultures on activity of Lactobacillus helveticus and Streptococcus thermophilus at temperatures near their maximum following heat shocking at 60° C. for thirty minutes

Culture	Incubation time and temperature	Number of transfers at respective temperature	Titratable acid of inoculating culture	Temperature of incubation following heat shocking	Time of incubation following heat shocking	Drop in pH
			Per cent	*° C.*	*Hours*	
L. helveticus	12 hrs. 37°	1	0.88	44	8	.60
	12 hrs. 40°	1	1.06	44	8	.35
L. helveticus	12 hrs. 37°	18	0.80	44	8	.09
	12 hrs. 40°	18	1.03	44	8	.59
L. helveticus	12 hrs. 37°	70	0.95	44	8	.23
	12 hrs. 40°	70	1.12	44	8	.77
L. helveticus (a)	12 hrs. 37°	96	0.89	44	9	.04
(b)	12 hrs. 40°	96	1.03	44	9	.45
(c)	14 hrs. 37°	96	1.00	44	9	.05
(d)	10 hrs. 40°	96	0.94	44	9	.43
Str. thermophilus	12 hrs. 37°	1	0.70	48	4	.73
	12 hrs. 40°	1	0.70	48	4	.68
Str. thermophilus	12 hrs. 37°	140	0.68	48	4	.56
	12 hrs. 40°	140	0.71	48	4	.31
Str. thermophilus	12 hrs. 37°	1	0.71	48	4	.88
	12 hrs. 37°	150	0.70	48	4	.51

(a) and (c) = Inoculated from mother culture carried at 37° C. for 48 days and transferred every 12 hours. Table shows incubation time and temperature of inoculating cultures.

(b) and (d) = Inoculated from mother culture carried at 40° C. for 48 days and transferred every 12 hours. Table shows incubation time and temperature of inoculating cultures.

tures following heating. Preliminary tests indicated that the most suitable incubation temperatures for such experiments were 44° for *L. helveticus* and 48° C. for *Str. thermophilus.*

The results, shown in Table 2, demonstrate certain differences in cultures of *L. helveticus* carried at 37° and 40° which were not brought out by incubation at lower temperatures following heating. After the first transfer of the mother cultures at 37° and 40°, the 37° appeared more resistant. After 18, 70 and 96 transfers, the 40° culture was without doubt the more active at 44° after the exposure to heat. The change in activity following heating was in the same direction as in previous experiments (*i.e.*, after numerous transfers the 40° appeared more heat resistant than the 37° culture). After the ninety-sixth transfer, the 37° mother culture was transferred to two inoculating cultures which were incubated at 37° for 12 and 14 hours, respectively. The 40° mother cultures were transferred to inoculating cultures which were incubated at 40° C. for 10 and 12 hours, respectively. The titrations of the 12 hour, 37° and the 10 hour, 40° cultures were 0.89 per cent and 0.94 per cent respectively. Those of the 14 hour, 37° and 12 hour, 40° cultures were 1.00 and 1.03 per cent. In spite of the close approximations in titratable acidities of mother cultures, the 40° culture lowered the pH after heating 0.43 and 0.45 as compared to 0.04 and 0.05 for the 37° culture. The greater maturity of the 40° inoculating cultures apparently was not responsible for their greater resistance and their activity at 44° following heating. Similar differences in heat resistance were obtained with stock cultures carried at 37° and 40°, respectively, and transferred every week.

The results obtained with *Str. thermophilus*, shown in Table 2, on the whole are similar to those obtained when an incubation temperature of 37° rather than 48° C. was employed following heat treatment.

When microscopic counts were made of cultures incubated at temperatures near their maximum following heating, irregular results were often obtained because of the formation of extremely long entangled chains and because unusually high numbers of the cells were gram-negative, due possibly to a change in staining properties after rapid growth at the high temperature.

Influence of time and temperature of incubation on activity of L. helveticus and Str. thermophilus at temperatures near their maximum following heat treatment

Previous experiments were concerned chiefly with the influence of incubation temperature and although certain allowance had been made for it, the fact soon became evident that greater emphasis must be placed on effect of time of incubation. The cultures of *L. helveticus* used for this experiment were being transferred daily, incubated for 14 hours at 37° or 40° and then placed at 10° for 10 hours until time for the next transfer. Those of *Str. thermophilus* were being transferred daily, incubated for 12 hours at 37° and 40° and then placed at 10° for 12 hours until time for the next

transfer. Results indicated that 40° cultures of *L. helveticus* and 37° cultures of *Str. thermophilus* were superior in each case. But as in earlier experiments, it was believed possible that an incubation time of 14 hours for *L. helveticus* and 12 hours for *Str. thermophilus* was not the optimum. Accordingly, fresh transfers of these two organisms, carried in the manner described, were used to prepare inoculating cultures which were then incubated at 37° and 40° for periods varying from 6 to 16 hours. The inoculating cultures were inoculated at such intervals that they might all be removed and heat shocked simultaneously. Titrations and pH determinations made of inoculating cultures indicated their relative maturity. Table

TABLE 3

Influence of time and temperature of incubation on activity of Lactobacillus helveticus at 44° C. following heat shocking at 60° C. for thirty minutes

Time and temperature of incubation of mother culture	Number of daily transfers of mother culture by this method	Time and temperature of incubation of inoculating culture	Titratable acid of inoculating culture	Drop in pH during first eight hours following heat shocking
			Per cent	
14 hrs. 37° 10 hrs. 10°	30	7 hrs. 37°	0.49	.00
	30	12 hrs. 37°	0.96	.29
	30	16 hrs. 37°	1.14	.26
14 hrs. 40° 10 hrs. 10°	30	7 hrs. 40°	0.64	.01
	30	12 hrs. 40°	1.10	.36
	30	16 hrs. 40°	1.26	.23
14 hrs. 37° 10 hrs. 10°	65	8 hrs. 37°	0.41	.03
	65	12 hrs. 37°	0.86	.42
	65	16 hrs. 37°	1.11	.46
14 hrs. 40° 10 hrs. 10°	65	8 hrs. 40°	0.56	.05
	65	12 hrs. 40°	1.04	.51
	65	16 hrs. 40°	1.25	.39

3 demonstrates that under the conditions employed in this experiment, the time of incubation definitely influenced the ability of *L. helveticus* to develop after heat shocking. Twelve and 16 hour cultures of *L. helveticus* were far more active following heat treatment than were the seven and eight hour cultures. As shown in Table 4, there was little measurable difference in heat resistance of cultures of *Str. thermophilus*, grown for periods of 6, 12, and 16 hours, respectively. In another trial there was little difference between 8 and 16 hour, 37° cultures of *Str. thermophilus*, and no significant difference between 8 and 16 hour, 40° cultures.

DISCUSSION

The results indicated that when *L. helveticus* was carried at temperatures of 30°, 35°, 37°, 40°, and 42° C. for the incubation periods commonly employed in actual practice for starter cultures of this organism and other related lactobacilli, the heat resistance of cultures incubated at temperatures of 37° and 40° was greater than that of cultures incubated above or

TABLE 4

Influence of time and temperature of incubation on activity of Streptococcus thermophilus at 44° C. following heat shocking at 60° C. for thirty minutes

Time and temperature of incubation of mother culture	Number of daily transfers of mother culture by this method	Time and temperature of incubation of inoculating culture	Titratable acid of inoculating culture	Drop in pH during first eight hours following heat shocking
			Per cent	
12 hrs. 37° 12 hrs. 10°	29	8 hrs. 37°	0.58	1.03
	29	12 hrs. 37°	0.68	1.02
	29	16 hrs. 37°	0.77	1.02
12 hrs. 40° 12 hrs. 10°	29	8 hrs. 40°	0.62	1.03
	29	12 hrs. 40°	0.72	.76
	29	16 hrs. 40°	0.77	.92
12 hrs. 37° 12 hrs. 10°	53	6 hrs. 37°	0.50	1.06
	53	12 hrs. 37°	0.71	1.05
	53	16 hrs. 37°	0.78	1.11
12 hrs. 40° 12 hrs. 10°	53	6 hrs. 40°	0.52	1.06
	53	12 hrs. 40°	0.73	1.08
	53	16 hrs. 40°	0.79	1.05

below these temperatures. When cultures were carried at 37° and 40° and transferred every 12 hours, it was found that the first transfer of the 37° cultures was fully as resistant as and sometimes more resistant to heat than the first transfer of the 40° culture. Titrations of the inoculating cultures showed that the 40° culture was more mature than the 37° culture. As the consecutive 12 hour transfers at the two respective temperatures were continued, the 40° culture gradually became the more resistant. These results indicated, then, that the higher temperature of incubation was responsible for the increased heat resistance. The 40° was more mature at the time of every transfer than the 37° culture and any advantage in heat resistance due to greater maturity may have accumulated over a number of successive transfers. On the other hand, the 40° culture was more mature at the first transfer yet less heat resistant than the 37° culture. This fact would suggest that maturity of the cultures was not a major factor in the development of differences in heat resistance between 37° and 40° cultures after repeated transfer. The results strongly indicate, then, that the higher incubation temperature was primarily responsible for greater heat resistance. Differences in numbers of organisms in the respective cultures before heating were not great enough to cause significant differences in the numbers surviving heat treatment.

Because the greater heat resistance of the 40° culture was not lost by one transfer at 37°, it would appear that the increased heat resistance of the 40° cells may have been permanent enough to survive several generations of growth at the lower temperature.

Incubation of heat shocked cultures at temperatures near their maximum emphasized the difference between 37° and 40° cultures of *L. helveti-*

cus. Results to appear in a later paper indicate that the number of active cells present in a culture largely govern its ability to develop at 44° C.

Streptococcus thermophilus, when carried at different temperatures by the same methods employed for *L. helveticus* demonstrated an entirely different behavior. Growth at 30°, 35° and 37° C. yielded cultures which were more active following heat treatment than were those grown at 40° and 42° C. When cultures of this organism were grown at 37° and 40° and were transferred every 12 hours, the 37° culture was more resistant than the 40°, both after one and after numerous transfers. These heat resistant studies were made on cultures which were about at the stage of maximum heat resistance. Titratable acidities of inoculating cultures indicated that possibly the greater amount of acid produced by the 40° as compared with the 37° culture had an injurious effect on the cells and therefore lowered their heat resistance. However, when cultures were incubated at 37° and 40° for periods varying from six to 16 hours, no great difference in heat resistance was obtained. This fact would minimize the influence of the greater amount of acid produced in a 12 hour incubation period at the higher temperatures on the heat resistance of *Str. thermophilus.* The results, as a whole, demonstrate that growth of *Str. thermophilus* at lower temperatures results in greater activity and heat resistance than growth at the higher temperatures used in these studies. *Str. thermophilus* has a higher maximum temperature and was apparently influenced less by the incubation time and type of milk in which it was grown than *L. helveticus.* This explains why it is easier to obtain active starter cultures of *Str. thermophilus* than of *L. helveticus.* The importance of the culture medium in developing active starter cultures will be discussed in a following paper.

The results obtained by incubation of cultures of *L. helveticus* and of *Str. thermophilus* at temperatures near their maximum following heat shocking explain why certain Swiss cheese starter cultures which show no outward differences in activity still vary so widely in their ability to develop at the high temperatures prevailing in Swiss cheese on the press. The high temperature effects a rather delicate balance in that it determines largely whether or not the starter organisms will develop at an early hour. If the culture is weak, it may not develop until the temperature falls to a favorable level. The consequent slow acid development in the curd on the press may result in serious defects in the cheese.

SUMMARY

1. When *L. helveticus* was carried for numerous transfers at different temperatures by methods similar to those commonly employed in handling starter cultures of this organism, those cultures carried at 37° and 40° were more active following heat treatment than were cultures carried at 30°, 35° and 42° C.

2. The 37° cultures of *L. helveticus* were more heat resistant than the 40° C. cultures after the first 12 hour transfer at the respective temperatures, but after numerous, successive 12 hour transfers at the respective temperatures, the 40° was more resistant than the 37° culture. This increased resistance of the 40° culture was not lost by one transfer at a lower temperature.

3. Cultures of *Str. thermophilus* carried for numerous successive transfers at different temperatures by the same methods used for *L. helveticus* showed greater heat resistance when grown at 30°, 35° and 37° than at 40° or 42° C. The 37° cultures were more active than were the 40° C. cultures of *Str. thermophilus* after both one and numerous successive 12 hour transfers at the respective temperatures.

4. Heat treatment in the usual manner, followed by incubation of the respective cultures at temperatures near their maximum and comparison of their subsequent rates of growth and acid production, emphasized the differences in activity following heat shocking of cultures of *L. helveticus* and *Str. thermophilus.*

5. Cultures of *L. helveticus* incubated at 37° and 40° C. for 12 to 16 hours were far more heat resistant than cultures incubated at 37° and 40° C. for seven and eight hours.

6. There was no marked difference in activity following heat treatment of cultures of *Str. thermophilus* incubated at 37° C. for periods varying from six to 16 hours, the range of incubation periods usually employed in growing starter cultures of this organism.

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July, 1938, page 335, line 17, should read as follows: "the preparation of 0.05 N KOH and C_2H_5ONa solutions."

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

561. Abstracts of Papers from the Proceedings of the Society of Agricultural Bacteriologists—England. Abstracts submitted to the JOURNAL OF DAIRY SCIENCE by I. G. Davis. Nat. Inst. Res. Dairy., Reading, England.

A. "The bacteriology of clotted cream"—E. L. CROSSLEY.

The two main factors controlling bacteria in clotted cream are bacterial multiplication during cooling and the handling operations during skimming, weighing and packing. Cooling should be as rapid as possible. The flora at this stage consists of spore forming bacilli, which may produce bitterness, but which are usually overgrown by acid forming organisms which enter later and finally predominate. Cleanliness and sterility of plant are essential to the production of a good cream.

B. "The bacteriology of spray dried milk powder"—E. L. CROSSLEY.

Powder from good quality milk contains far fewer bacteria than that from poor quality milk although similar types are found. The number varies from thousands up to several millions per g., thermoduric cocci forming the bulk of the flora. *B. subtilis* type aerobic spore-formers are always present. Yeasts, moulds, anaerobes (*Cl. welchii*) and heat-resisting coli are sometimes present. Acid-forming types nearly always predominate. The death rate appears to depend on the types of organisms present.

C. "The enumeration of bacteria in cheese"—J. HARRISON.

It was found that emulsions of ripe Cheddar cheese prepared with hand emulsifiers gave plate counts nearly ten times as high as emulsions prepared by grinding with sand in a mortar. When young cheese were used, counts about 2 or 3 times as high were obtained. Different emulsifiers gave similar counts. It is essential to take several plugs if an accurate idea of the bacterial count of a cheese is to be obtained. A device for random selection of colonies from a plate is described.

D. "A note on the estimation of volatile acids of cheese"—E. R. HISCOX AND J. HARRISON.

Lactic acid and CO_2 are responsible for errors in the estimation of volatile acids in cheese. If the fat and the ether extract are used the lactic acid error is negligible. The CO_2 may be removed from the distillate by drawing CO_2-free air through it for 20 minutes.

E. "The incidence of milk samples yielding acid and gas in McConkey broth at 30° C."—C. D. OXLEY.

A test of 205 samples of milk by the methylene blue test, the "presumptive coli" test at 37° and at 30° C. showed that the last was responsible for more failures than the others. This test picks out more "poor" samples than any other.

F. "Statistical examinations of the results of a co-operative experiment on methylene blue reduction tests and bacterial plate counts"—H. BARKWORTH AND A. T. R. MATTICK.

A comparison of the plate count and methylene blue reduction test showed that 87 per cent of samples failed or passed both tests. The correlation between plate count and reduction time was lower in winter, although here a high correlation was found with high plate count. Four laboratories testing common samples agreed on plate counts and reduction times at 37° C., but gave differences with the keeping quality test and the reduction test at 15.5° C.

G. "Some experiments on the value of the Methylene Blue Test and the Resazurin test for the grading of milk during winter"—S. B. THOMAS.

A comparison of the old method of carrying out the methylene blue test with the Wilson modification showed that half hourly inversion decreased the reduction time on the average by 5 hours.

The mean reduction time for samples held in the refrigerator at 40° F. overnight was 3½ hours longer than for duplicates held overnight at 60° F.

The variations in the reduction times of duplicate tubes observed by one worker increased in number and magnitude as the reduction times increased. Quintuplicate tests by one worker, taking readings every five minutes, gave a mean coefficient of variation of reduction times as low as 0.7 per cent, whereas the mean C.V. obtained by four workers using separate water baths was 6.5 per cent, and for duplicate tests at each of four laboratories 9.4 per cent. A comparison of the Resazurin test with the methylene blue test showed that the former is worthy of further investigation.

H. "The effect of storage time and temperature on the methylene blue reduction test for milk"—M. BRAZ AND W. A. HOY.

The importance of time and temperature of storage is emphasized. In practice wide variations in temperature may occur. A series of tests showed that storage for 12 hours at 65° F. is equivalent to 18 hours at 60° F. A temperature of 40–50° F. has a considerable retarding effect on the test, but a storage temperature of 60° F. reveals a milk in its true character.

I. "A comparison between the methylene blue reduction test and the plate count with changing conditions of milk production and with changing conditions of storage of milk samples before testing"—A. A. NICHOLS AND C. J. JACKSON.

A study of the comparative sensitivity of the plate count and methylene blue reduction test to bad methods of production is described. The latter is more sensitive if the samples are held at 72° F. and (for dirty conditions) at room temperature. A short holding followed by refrigeration reveals very little difference. Testing immediately after milking or after storage at low temperatures showed that the plate count provided the better index.

J. "A comparison of the results of different methods of analysis and of sampling of milk during 1937"—M. E. PIRRIE.

The present Accredited Standard was found to be more severe than the old Grade A standard on account of the holding of small samples at atmospheric temperature for 12 or 18 hours. Both plate count and methylene blue tests give similar results if samples are held under identical conditions. Icing of samples during transit and holding is recommended.

K. "Mastitis in relation to the methylene blue reduction test"—J. G. DAVIS AND J. MCCLEMONT.

Mastitis has a much more marked effect on plate count than on methylene blue reduction time. Methylene blue is toxic to *Str. agalactiae* so that it is unlikely that this organism will either proliferate or reduce the dye in the test. The slightly shorter reduction times due to mastitis may be attributed to the higher cell content and to the increased flora. Holding the samples at 4° C. or 15.5° C. did not appear to increase the differentiation between infected and non-infected samples. The methylene blue test is of no use for the detection of mastitis.

L. "Indirect methods of mastitis diagnosis"—M. ZEIN-EL-DINE AND S. J. ROWLAND.

Clinical examination (by palpation) could only detect 7% of the cases. "Clots" or flakes of the fore-milk, and brom-thymol-blue papers could detect 12 and 14% respectively of the positive quarters. No "false positives" could be found with these three tests.

The brom-cresol-purple papers could detect 61% of the positive cases and classed 27% as doubtful, and only 12% could be passed as normal; but, on the other hand, it classed 16% of the negative cases as positive and 22% as doubtful.

The centrifuge deposit test could detect 55% of the positive cases and classed 23% as doubtful, while classifying only 3% as false positives. This

test gave 25% false doubtfuls. The catalase test was useless as it detected less than half of the positive cases, and gave many false positives and false doubtfuls.

The chloride test (digestion method) could detect 57% of the positive cases and gave no false positives. The casein number method detected 91% of the positive cases and gave 8% false positives.

The solids-not-fat in the fat-free milk is a good test for mastitis (solids-not-fat in fat-free-milk lower than 8.76%) 88% of the low solids-not-fat milks examined were found to be infected. "Physiologically low solids-not-fat milks" were thus found to be comparatively rare.

M. "The diagnosis of *Str. agalactiae* in a liquid selective medium"—S. J. EDWARDS.

A highly selective medium having the following composition: Lemco broth (pH 7) 1,000 ml., dextrose 5 g., crystal violet (purified) 0.1%, 1 ml., sodium azide 0.1 g., has been found to give good results. It is recommended for checking the clean section of a herd after a blood agar plate diagnosis has been carried out.

N. "Further studies of the aerobic spore-forming bacilli"—T. GIBSON AND L. E. TOPPING.

A provisional key to assist in the identification of the commonest species of aerobic spore-forming bacilli is presented. Barritt's modification of the Voges-Proskauer test and the production of acid or acid and gas from glucose are the main diagnostic criteria utilized. The key does not include obligate thermophiles or a few relatively uncommon organisms.

O. "The production of lactic acid from substances other than sugar by *L. casei*"—C. C. THIEL.

A strain of *L. casei* has been found to produce more lactic acid in pancreatic and peptic casein and milk digests than is accounted for by the sugar utilized. Also, in pancreatic and peptic casein digests to which no sugar is added, a considerable production of lactic acid (from 0.10 to 0.28 g. per 100 ml.) occurs. A casein suspension does not support growth; added alanine does not increase the lactic acid production in the digests.

Discrepancies between sugar utilized and acid produced do not occur in separated milk and separated milk plus yeast.

P. "The effect of sterilization upon sugars"—J. G. DAVIS AND H. J. ROGERS.

Autoclaving sugar solutions for 30 minutes at 120° C. is very much more destructive than "momentary autoclaving," which is only slightly more

destructive than the usual intermittent steaming method. Momentary autoclaving, unlike intermittent steaming, can be relied upon to sterilize media in tubes. Differences in fermentation tests due to heating the sugar in, or apart from, the medium do not seem to be significant. As a standard technique momentary autoclaving of all sugar broths at pH 6.6 is recommended.

BREEDING

562. Extending the Use of a Proved Sire. ALLAN K. BROWN, Guernsey Breeders' Journal, 54, 705, October 15, 1938.

Dr. Allan K. Brown, owner of Brown Ranch, Capitola, California, has practiced artificial insemination in his herd since 1937 and he says that the advantages of this method are:

1. Increased number of progeny obtainable from an ageing and proven sire.
2. Relief from overuse, particularly an old bull.
3. Overcoming physical defects, injuries, or disease in both female and male, such as adhesions in the bull, and cervitis in the cow.

The formula for diluting mediums is:

Water 100 CC.
Glucose 3%
With a pH of 7.5.

When the records indicate that several cows are due to come in heat within a period of one or two days the cow(s) coming in heat first are held over a day or two until the others come in heat. The first cow(s) of the group may then no longer accept the bull. However, experience has shown that conception will occur with considerable regularity if the cow is artificially inseminated at this time. One of the cows in heat is bred, the semen removed, diluted and a one cubic centimeter portion discharged into the opening of the cervix of the other cows by an inseminating syringe. Eight cows have been successfully bred by holding them over three days.

His results have been very satisfactory, as out of forty-seven cows bred, forty of them conceived the first time. Eighty per cent of the animals in the breeding herd of 125 females were bred to one bull. L.R.L.

FEEDS AND FEEDING

563. Legume and Grass Silages. O. M. CAMBURN, H. B. ELLENBERGER, J. A. NEWLANDER AND C. H. JONES. Vermont Agricultural Experiment Station Bul. *434*, May, 1938.

The data from one season's trials indicate that:

Legumes and grasses may be successfully ensiled either with or without molasses provided their dry matter contents range between 30 and 40 per cent and preferably as near to 35 per cent as may be.

A dry matter content of less than 30 per cent favors putrefactive fermentation, especially if molasses is not added; one of over 40 per cent tends to prevent tight packing in the silo and induces heating and spoilage.

The ensiling of legumes or grasses is not advisable under ordinary farm conditions unless molasses or acid are added, for the reason that it is hard to control the dry matter content of silage.

The addition of two per cent (40 pounds per ton) of molasses to ensiled timothy and of three or four per cent (60 to 80 pounds per ton) to ensiled alfalfa tends to induce fermentation and to decrease the likelihood of spoilage due to the presence of too much or too little moisture. The odor and palatability of such silages are improved when molasses is used and their feeding value may be slightly enhanced.

The minerals, proteins, and total nutrients are better preserved by ensiling than by ordinary haying methods.

Close packing and exclusion of air are essential to the proper preservation of grass silages, especially those somewhat low in moisture due to wilting after cutting or to maturity of the crop.

An addendum report attached to this bulletin sets forth the results secured in an extensive survey of practical operations throughout the Northeast.

O.M.C.

564. Digestibility of Alfalfa, Timothy, and Soybeans as Silages and as Hays. J. A. NEWLANDER, H. B ELLENBERGER, O. M. CAMBURN AND C. H. JONES. Vermont Agr. Exp. Sta. Bul. *430*, March, 1938.

Coefficients of digestibility have been determined with dairy cows for 41 lots of silages and hays made from the 1936 crops of alfalfa, timothy and soybeans. Silages were made both with and without the addition of molasses to freshly cut and slightly wilted cuttings. Hays were made by natural sun curing and by artificial drying. The alfalfa and the timothy were cut at two stages of maturity.

On a dry matter basis most of the hays carried less ether extract and ash than the silages and more nitrogen-free extract. The artificially dried hays contained less fiber than the silages and sun cured hays.

The digestion coefficients of the hays, especially those which were artificially dried, were slightly higher in nitrogen-free extract but significantly lower in ether extract, especially in the case of the sun cured hay. Crude fiber and nitrogen-free extract seemed slightly more digestible in the silages to which molasses was added than in those not so treated.

On a dry-matter basis the silages carried slightly more digestible protein than did the hays. The artificially dried hays carried the most total digestible nutrients, followed in order by the molasses silages, the silages without molasses treatment and the sun cured hays, the latter two being about equal.

O.M.C.

565. The Mineral Needs of Farm Animals. Departments of Animal Industry and Dairy Husbandry, OHIO AGR. EXP. STA., Wooster, O. Ohio Exp. Sta. Spec. Circ. *49*. 1937.

The Vitamin Needs of Farm Animals. IBID. Spec. Circ. *52*. 1938.

These two companion circulars present in popular style the requirements of livestock for the various minerals and vitamins and make practical recommendations as to how these requirements can be met. W.E.K.

566. Nutritional Requirements of Pregnant and Lactating Rats Studied by the Self-selection Method. CURT. P. RICHTER AND BRUNO BARELARE JR., Henry Phipps Clinic, Johns Hopkins Hospital, Baltimore, Md. Proc. Amer. Physiol. Soc., Amer. J. Physiol., *123*, 1, p. 170, July, 1938.

Ten female rats were allowed to select their food ad libitum from the following 11 substances; casein, sucrose, olive oil, sodium chloride, dibasic sodium phosphate, calcium lactate, potassium chloride, dried baker's yeast, cod liver oil, wheat germ oil, and water.

These animals gave birth to normal litters and nursed most of them until they were weaned 25 days later. The changes in appetite for the 11 substances were strikingly similar in the 10 animals. It was observed that the animals voluntarily ingested large amounts of fat; in fact, over half (52.6%) of the calories were furnished by fat. The fat consumption increased during pregnancy and lactation, supplying 65 per cent of the calories at the end of lactation. Protein furnished 22.3 per cent of the calories before mating, 28.1 per cent at the end of gestation, and 23.8 per cent at the end of lactation. The appetite for sodium chloride, dibasic sodium phosphate, and calcium lactate showed striking increases during pregnancy and lactation. The appetite for wheat germ oil and cod liver oil remained essentially the same. The number of calories increased from 45.3 per day before mating to 58.8 at the end of gestation and to 118.3 at the end of lactation.

The fact that the animals grew and reproduced with as great success as on the McCollum diet, in spite of lower solid food and caloric intake, is evidence of the efficiency of the self selection method in the rat. D.L.E.

567. The Effect of Different Per Cents of Protein in the Diet in Successive Generations. JAMES R. SLONAKER, Dept of Physiology, Stanford University, California, Amer. J. Physiol. *123*, 2, p. 526, August, 1938.

Five groups of rats and their succeeding six generations were fed rations containing the following per cents of protein, fats and carbohydrates respectively: I—10.3, 12.2, 77.5; II—14.2, 14.2, 71.6; III—18.2, 15.9, 65.9; IV—22.2, 17.8, 60.0; V—26.3, 19.7, 54.0.

The animals comprising these successive generations were all kept in the same room and under the same environmental conditions as their ancestors. Although the animals in group I were smallest at birth and grew slower (both sexually and in skeletal growth) than any of the other groups, their life span was unusually long. Group V grew the fastest up to 70 days of age and attained the greatest final length. On the other hand, the greatest prenatal development took place among the rats fed 18.2 per cent protein. They also proved to be the best mothers.

Protein in the diet equal to, or in excess of, 18.2 per cent interfered with reproduction by increasing sterility. Such a diet also tended to shorten the breeding period and increase the size of the kidneys. In other words, the metabolic processes were quite generally speeded up by increasing the percentage of protein in the ration. The amounts of protein in the rations studied in these trials had no effect on the number of young born or on the sex ratio. D.L.E.

FOOD VALUE OF DAIRY PRODUCTS

568. The Value of Milk in the Diet. W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, O. Ohio Bimonthly Bull. XXIII, No. 194, Sept.-Oct., 1938.

This is a popular discussion of the food value of milk and is suitable for a 12-minute radio talk. The story builds up to make the point that the ultimate goal for milk consumption should be a pint a day for adults and a quart a day for children. W.E.K.

ICE CREAM

569. The Frozen Food Industry. IVAN C. MILLER, McGraw-Hill Pub. Co., New York, Ice Cream Trade J., *34*, 9, p. 31, Sept., 1938.

Ice cream manufacturers are watching the development of the frosted food industry. Today he is not an important factor in the distribution of frozen foods. There is every reason to expect that in the future an important portion of retail frozen food distribution will be through the channels of the ice cream manufacturers. W.H.M.

570. Manufacture of Sherbets and Ices. W. H. MARTIN, Kansas State College, Manhattan, Kans. Ice Cream Trade J., *34*, 9, p. 12, Sept., 1938.

This article discusses the problems involved in the manufacture of sherbets and ices. Information is presented on sugar content, stabilizer, color, flavor, use of milk products, overrun, and storage. Formulae for sherbets and ices are given. W.H.M.

571. Triple Scoops. MALCOLM PARKS. Ice Cream Trade J., *34*, 8, p. 13, Aug., 1938.

The use of three No. 30 scoops of ice cream or sherbet in cones and other ice cream dishes has been found by many ice cream manufacturers to be an effective means of increased volume of sales. Mr. Parks presents dealers' cost sheet to show the profit possibilities in this type of merchandising and also gives directions for the preparation and sale of the various items made with three scoops of ice cream. W.H.M.

572. Frozen Food. PAUL C. TRIMBLE. Ice Cream Trade J., *34*, 8, p. 8, August, 1938.

Ice cream manufacturers who are considering the possibility of distributing frozen foods will be interested in this article which describes the experience of the Southern Dairies in the frosted foods field in the southern states. W.H.M.

573. Stabilizers and Their Use in Ice Cream. L. M. LAMPERT, State Dept. of Agriculture, Sacramento, Calif. Ice Cream Trade J., *34*, 7, p. 19, July, 1938.

A definition and classification of stabilizers is given, together with a description of the properties of the various products used in the stabilization of ice cream. The author states that no one can object to their use, provided that they meet reasonable requirements for efficiency, wholesomeness and purity. W.H.M.

574. Increased Sanitation in the Dispensing of Ice Cream. F. W. FABIAN, Michigan State College, East Lansing, Mich. Ice Cream Trade J., *34*, 6, p. 20, June, 1938.

Surveys made by Fabian and others of conditions which surround the sale of ice cream indicate the need for ice cream manufacturers to pay more attention to their product after it leaves the plant. He recommends schools of instruction for those serving ice cream. Measures which will reduce the disease hazard in connection with the sale of ice cream are as follows:

1. Require that all scoops or other implements used in dishing ice cream be kept in water flowing at such a rate so as practically to eliminate the possibility of reproduction and growth of bacteria.

2. Require that all the dishes, silverware, and glassware used in serving ice cream be properly washed. These principles hold for either hand or mechanical washing. Water at a temperature of 140° F. to which has been added one per cent of a good detergent is the lowest best practical temperature to use. After the dishes, glasses, and spoons are washed, they should be rinsed in water at a temperature of not less than 170° F.

3. A bacterial standard of 5000 per gram for flavoring syrups and fruits is suggested.

4. The dispenser should be free of contagious disease and should be clean and neat in his personal appearance.

5. The use of individual package goods should be encouraged by all those interested in producing and serving ice cream. This will do away with most of the objectionable sanitary features which one now observes at many serving places. W.H.M.

575. The Influence of Temperature Upon the Extent to Which Freezing Occurs in Ice Cream. W. C. COLE, Div. of Dairy Industry, Univ. of Calif., Davis, Calif. Ice Cream Trade J., *34*, 6, p. 15, June, 1938.

The amount of ice formed at different temperatures in ice cream, ice milk, and sherbets was measured by using a dilatometer. Known weights of the samples to be tested were admitted into the apparatus in such a manner that changes in volume of the sample could be determined. By taking into account the difference in volume before and after freezing at a given temperature and also considering the increase in volume accompanying the change of one gram of water to ice it was possible to calculate the amount of ice formed in the sample at that temperature. The authors summarize their results as follows:

1. Taking into account the changes in volume accompanying the crystallization of ice from solutions it has been possible to measure with a dilatometer the percentages of ice formed in ice cream and related products from 25° C. (13° F.), or slightly lower, to temperatures corresponding to the freezing points of the various samples studied.

2. The results obtained by this method show that at corresponding temperatures the percentages of water frozen in ice milk and ice cream are essentially the same for samples included in this study. Considerable variation occurred, however, in the percentages of ice in these samples depending upon the proportion of water they contained.

3. The results obtained for ices, varied considerably from those obtained for ice cream at corresponding temperatures, both as to the percentages of water frozen and the percentages of ice in the samples.

4. Presumably the data presented can be used as an indication of the extent to which freezing occurs in the commercial manufacture of ice cream and related products. W.H.M.

MILK

Abstracts of interest are 561, 568, 576 and 577.

MISCELLANEOUS

576. Safety Improvement Thru Drivers Award System. EDGAR G. QUESNEL, the Borden Company, Ice Cream Trade J., *34*, 9, 13, Sept., 1938.

The Borden Company has experienced a 25 per cent improvement in the safe operation of their vehicles during the past five years by using an award system for their drivers. The awards are usually lapel emblems, badges or certificates and sometimes cash. Accomplishments which may be expected by everyone participating in an Award System of this kind are always worthwhile. They result in good industrial and public relations, better transportation service, lower costs, better work, fewer interruptions, few accidents, less personal injury and property damage, minimum of public liability claims, and most important of all the conservation of the most valuable asset the company has—the life, happiness, ambitions and future accomplishments in the field of constructive service—the employee himself.

W.H.M.

577. Cold Storage Lockers. P. EDWIN THOMAS. Ice Cream Trade J., *34*, 7, p. 21, July, 1938.

"Practically unknown five years ago, some 2,000 cold storage locker businesses are reported in operation in almost half the States of the Union, with an investment of nearly $25,000,000." A floor plan for a 320 locker plant 35 feet wide by 70 feet long is illustrated. The cost of such a plant is estimated at $25 to $50 per locker and according to the figures presented, it would be possible for the plant to yield a return on investment of 10 per cent.

W.H.M.

578. Index of Research Projects, Works Progress Administration.

The results of some 2,000 research projects carried on as part of the federal work relief program are summarized briefly in a digest and index which has been published by the Works Progress Administration. This volume of 291 pages contains a concise statement of the principal conclusions of each study and an alphabetical subject index to the contents. The reports on these projects touch upon nearly every field of natural and social science and many of them have appeared in the form of articles in scholarly journals. However, several hundred of the reports summarized in this index are in manuscript form, and arrangements have been made with the American Documentation Institute whereby microfilm copies of the original reports will be furnished at nominal rates for the use of research specialists. A small edition of this volume has been prepared for distribution to the larger public and university libraries, where it will be available for reference, and for government departments, industrial concerns and research

foundations. A limited supply of copies of this Index of Research Projects are still available. Requests should be addressed to the Works Progress Administration in Washington. H.K.H.

PHYSIOLOGY

579. Concerning the Metabolism of Fat and Carbohydrate. JOSEPH L. DONNELLY, Fort Thomas, Ky. Amer. J. Physiol. *124*, 1, p. 126, October, 1938.

From a selected group of data published by other workers, the author draws some very important inferences on the relation of total acidity of the body to fat and carbohydrate metabolism. He points out that as a general mode of behavior there is an increase in the size of the globules of fat in the tissues with any increase in the acidity of the animal. This increase in size of fat globules results in a loss of reactivity which parallels the decrease in dispersion of the fat. Moreover, the activity of lipase and bile is diminished by acid. After pointing out the apparent relationship between the concentration of sugar and fat in the blood, the author draws the conclusion that "the size of the tissue fat globule and the concentration of fat and carbohydrate in the blood vary directly while the utilization of fat and carbohydrate vary inversely with the total acidity of an animal."

The statement of Professor Denning (Research and Progress 4: 41. 1938) that soya bean meal induces alkalasis is of interest here. D.L.E.

580. The Effect of Pregnancy and Lactation on Growth in the Rat. H. H. COLE AND G. H. HART, College of Agriculture, University of California, Davis. Amer. J. Physiol. *123*, 3, p. 589, September, 1938.

The authors show that pregnancy stimulates skeletal and tissue growth in the rat beyond that found in non-bred littermate controls. The excess growth during pregnancy is accompanied by, and presumably dependent upon, an increased food consumption manifested by the second day after conception. It is postulated that the copulatory act brings about a nervous stimulation of the anterior pituitary, resulting in the secretion of one or more hormones involved in inducing an increased appetite.

The excess gains made by the pregnant rats remain fairly constant for the first six pregnancies, after which further pregnancies have less effect. The excess gains are made, for the most part, during pregnancy although rats suckling four to six young continue to gain as rapidly as non-bred controls. D.L.E.

581. The Ratio of Arterio-venous Differences of Certain Substances to Quantities Secreted by the Mammary Gland. J. C. SHAW AND

W. E. PETERSEN, Department of Dairy Husbandry, University of Minnesota, Minneapolis, Minn. Proc. Amer. Physiol. Soc., Amer. J. Physiol. *123*, 1, p. 183, July, 1938.

Arterio-venous differences were determined on bloods drawn from the internal iliac and external pudic arteries and the subcutaneous abdominal milk veins for calcium, glucose, lactic acid, fat and amino acids.

The ratio of volume of blood flow traversing the gland to the amount of milk secreted was 1 : 387 when calculated from the blood calcium differences and the calcium in the milk. The ratio of the combined glucose and lactic acid differences to the lactose in the milk was 1 : 390 which is in good agreement with the calcium ratio. The differences in neutral fat indicated a ratio of 344 volumes of blood per volume of milk. The amount of amino acids removed by the gland are inadequate to account for more than 40 per cent of the proteins of the milk on this basis. The essential data follow.

	# of analyses	Arterial venous differences	Per cent in milk	Ratio
Amino-acid nitrogen	15	0.46 mgm. %	500 (total nit.)	1 : 1087
Glucose-lactic acid	17	12.44 "	4860 (Lactose)	1 : 391
Fat	39	11.63 "	4000	1 : 344
Calcium	20	0.31 "	120	1 : 387

D.L.E.

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Correspondence regarding business policies of the Journal should be addressed to the Secretary-treasurer, R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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The American Dairy Science Association was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

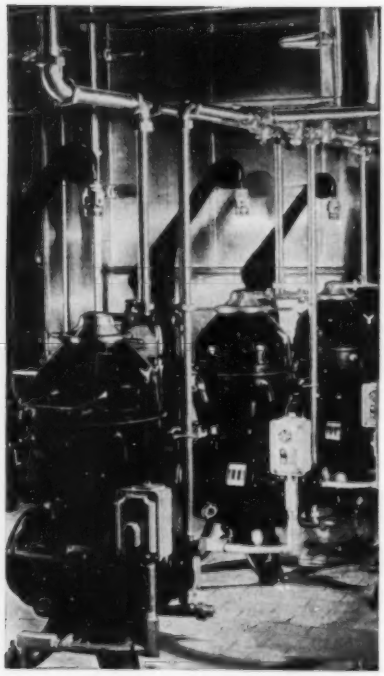
Membership shall consist of two kinds: (1) active, (2) associate.

The qualifications for membership in the two classes are as follows: (a) Any person is eligible to active membership who is formally announced by an Agricultural College, or Experiment Station, or by the Bureau of Dairying of the United States Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or (b) anyone filling a position of responsibility connected with the dairy industry and who has had a college or university training in technical science, or anyone filling a responsible position in the industry of a professional character requiring a technical knowledge of dairying of a high order.

Any person is eligible to associate membership who is regularly enrolled in a collegiate course in a College of Agriculture and who is specializing in dairying. Associate membership is attained by election to membership in a local chapter of *The American Dairy Science Association.*

The dues are $5.00 a year for active membership. Correspondence regarding membership and dues should be addressed to R. B. Stoltz, Ohio State University, Columbus, Ohio.

Clarification helps produce superior milk in modern plants



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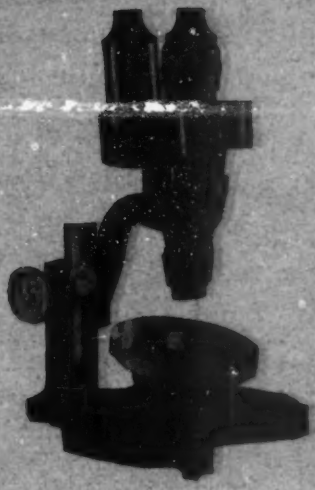

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The Spencer Low Power Binocular Microscope gives a vivid stereoscopic image of extraneous materials in butter or cheese.

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